

## 207 Human Secreted Proteins

This application is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of copending International patent application Serial No: PCT/US01/05614 (in English), filed February 21, 2001, which is hereby incorporated by reference, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Patent Application Serial Nos. 60/184,836 filed February 24, 2000 and 60/193,170 filed March 29, 2000, both of which are hereby incorporated by reference, and this application is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of copending United States patent application Serial No. 09/205,258 filed December 4, 1998, which is hereby incorporated by reference, and which claims benefit under 35 U.S.C. § 120 of International patent application No.: PCT/US98/11422 (in English), filed June 4, 1998, which is hereby incorporated by reference, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Applications, all of which are hereby incorporated by reference:

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### ***Field of the Invention***

This invention relates to newly identified polynucleotides, polypeptides encoded by these polynucleotides, antibodies that bind these polypeptides, uses of such polynucleotides, polypeptides, and antibodies, and their production.

### ***Background of the Invention***

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

### *Summary of the Invention*

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

### *Detailed Description*

#### **Definitions**

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA

preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID

NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking

reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in

a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or

5 may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of  
10 covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

15 (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

20 "SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the  
25 present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not  
30 more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

## **Polynucleotides and Polypeptides of the Invention**

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

This gene is expressed primarily in melanocytes and, to a lesser extent, in testes, ovary, kidney and other tissues.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of neural crest derived cells including pigmentation defects, melanoma, reproductive organ defects, and defects of the kidney . Similarly, polypeptides and  
10 antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skin, reproductive, and renal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. melanocytes, testes, ovary,  
15 kidney, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 The tissue distribution in melanocytes indicates that the protein product of this gene is useful for treating disorders that arise from alterations in the number or fate of neural crest derived cells including cancers such as melanoma and defects of the developing reproductive system.

Many polynucleotide sequences, such as EST sequences, are publicly  
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
30 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2512 of SEQ ID NO:11, b is an

integer of 15 to 2526, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 2

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

ENMICVKCLPQYPEHSKHV (SEQ ID NO:487). Moreover, fragments and variants

10 of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also  
15 encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in infant brain and fetal lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
20 and for diagnosis of diseases and conditions which include, but are not limited to, developmental disorders of the brain or lung. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous and pulmonary systems,  
25 expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, lung, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in  
30 healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in infant brain and fetal lung indicates that the protein product of this gene is useful for treating or diagnosing disorders associated with



abnormal proliferation of cells in the Central nervous system and developing lung. Furthermore, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1117 of SEQ ID NO:12, b is an integer of 15 to 1131, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: ARVAFHLICRYILPTVYCHV (SEQ ID NO:488). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are

encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in breast lymph node, and to a lesser extent,  
5 in ovarian cancer and chondrosarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune responses such as inflammation or immune surveillance for tumors. This  
10 gene may be important for inflammatory responses associated with tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be  
15 routinely detected in certain tissues or cell types (e.g. lymph nodes, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 251 as residues: Lys-45 to Val-50, and/or Lys-69 to Arg-76.

The tissue distribution in breast lymph node indicates that the protein product of this gene is useful for the treatment or diagnosis of immune responses, including those associated with tumor-induced inflammation. Furthermore, given the tissue  
25 distribution, the gene product may also be involved in lymphopoiesis. In a case such as this, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 927 of SEQ ID NO:13, b is an integer of 15 to 941, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

#### 10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 4**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

ELVESPGAAGNSARSGNVVC (SEQ ID NO:489). Moreover, fragments and

15 variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in T-cells and T-cell lymphomas.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunological diseases involving T-cells such as inflammation, autoimmunity, and cancers including T-cell lymphomas. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of T-cells and other cells of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues)

or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution in T-cells and T-cell lymphomas indicates that the protein product of this gene is useful for diagnosing and treating T-cell based disorders such as inflammatory diseases, autoimmune disease and tumors including T-cell lymphomas. Furthermore, the tissue distribution indicates that the polypeptides or polynucleotides are useful for the treatment, prophylaxis, and diagnosis of immune  
10 and autoimmune diseases, such as lupus, transplant rejection, allergic reactions, arthritis, asthma, immunodeficiency diseases, leukemia, and AIDS. Additionally, expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets  
15 for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 829 of SEQ ID NO:14, b is an integer of 15 to 843, where both a and b correspond to the positions of nucleotide  
25 residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

30           This gene is expressed primarily in activated monocytes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include, but are not limited to, inflammation, autoimmunity, infection, or disorders involving activation of monocytes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 253 as residues: Asp-19 to Arg-31.

The tissue distribution indicates that the protein product of this gene is useful for diagnosing or treating diseases that result in activation of monocytes including infections, inflammatory responses or autoimmune diseases. Furthermore, expression of this gene product in monocytes also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1004 of SEQ ID NO:15, b is an integer of 15 to 1018, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 6

The translation product of this gene shares sequence homology with terminal deoxynucleotidyltransferase which is thought to be important in catalyzing the elongation of oligo- or polydeoxynucleotide chains.

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:  
FKKLVNPRXQGIRHEEEAVSWQERR (SEQ ID NO:490). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
10 these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

15 This gene is expressed primarily in activated human neutrophils, and to a lesser extent in T-cells, primary dendritic cells and bone marrow cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
20 cancers, particularly those of the blood such as leukemia and deficiencies in neutrophils such as neutropenia, and immune system disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the  
25 cardiovascular and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
30 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils and other immune cells, combined with the homology to terminal deoxynucleotidyltransferase indicates that the protein product of this gene is useful for the treatment and differential diagnosis of acute leukemias. Alternatively, this gene may function in the proliferation of neutrophils and be useful as a treatment for neutropenia, for example, following neutropenia as a result of chemotherapy. Additionally, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of hematopoietic disorders. This gene product is primarily expressed in hematopoietic cells and tissues, suggesting that it plays a role in the survival, proliferation, and/or differentiation of hematopoietic lineages. This is particularly supported by the expression of this gene product in bone marrow, which is a primary site of definitive hematopoiesis. Expression of this gene product in T cells and primary dendritic cells also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 647 of SEQ ID NO:16, b is an integer of 15 to 661, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 7**

The translation product of this gene exhibits a reasonable homology to the human chorionic gonadotropic (HCG) analogue-GT beta-subunit as disclosed in U.S. Patent No. 5,508,261 and PCT Publication No. WO 92/22568. There is a high

degree of conservation of the structurally important cysteine residues between these proteins.

This gene is expressed primarily in IL-1 and LPS induced neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the immune system, including inflammatory diseases and allergies.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the treatment/diagnosis of diseases of the immune system, since expression is primarily in neutrophils, and thus the translation product of this gene may be useful as a growth factor for the differentiation and/or proliferation of neutrophils for the treatment of neutropenia, for example following chemotherapy.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 539 of SEQ ID NO:17, b is an integer of 15 to 553, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.



## FEATURES OF PROTEIN ENCODED BY GENE NO: 8

This gene is expressed primarily in IL-1 and LPS-induced neutrophils.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the immune system, including inflammatory diseases and allergies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
10 providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid  
15 and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 256 as residues: Ser-14 to Pro-22, and/or Leu-43 to Val-53.

20 The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the treatment and diagnosis of diseases of the immune system, since expression is primarily in neutrophils, and thus the translation product of this gene may be useful as a growth factor for the differentiation and/or proliferation of neutrophils for the treatment of neutropenia, for example following chemotherapy.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
30 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 855 of SEQ ID NO:18, b is an

integer of 15 to 869, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 9

When tested against Jurkat cell lines, supernatants removed from cells expressing this gene activated the NF-kB transcription factor. Thus, it is likely that the protein encoded by this gene activates Jurkat cells by activating a transcriptional factor found within these cells. Nuclear factor kB is a transcription factor activated by a wide variety of agents, leading to cell activation, differentiation, or apoptosis. Reporter constructs utilizing the NF-kB promoter element are used to screen supernatants for such activity.

This gene is expressed primarily in IL-1 and LPS induced neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the immune system, including inflammatory diseases and allergies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 257 as residues: Tyr-22 to His-35.

The tissue distribution in neutrophils, combined with the biological activity data suggest that the protein product of this gene is useful for the treatment and/or diagnosis of diseases of the immune system, since expression is primarily in

neutrophils, and thus the translation product of this gene may be useful as a growth factor for the differentiation and/or proliferation of neutrophils for the treatment of neutropenia, for example following chemotherapy.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 945 of SEQ ID NO:19, b is an integer of 15 to 959, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 10**

This gene is expressed primarily in activated T-cells and to a lesser extent in endothelial cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune dysfunctions including cancer of the T lymphocytes and autoimmune disorders and inflammation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample

taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in activated T-cells indicates that the protein product of this gene is useful for the treatment and/or diagnosis of immune disorders, particularly of T-cell origin, and may act as a growth factor for particular subsets of T-cells such as CD4 positive cells, which would make this a useful therapeutic for the treatment of HIV and other immune compromising illnesses. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of AIDS or other immune compromising diseases (e.g. by boosting immune responses).

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1432 of SEQ ID NO:20, b is an integer of 15 to 1446, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 11**

The gene encoding the disclosed cDNA is thought to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in fetal tissues, such as liver/spleen and brain, as well as in placental tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for the diagnosis of many developmental abnormalities. Similarly, polypeptides

and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developing fetus, expression of this gene at significantly higher or lower levels may be routinely detected in certain

5 tissues or cell types (e.g. fetal, placental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 The tissue distribution in fetal tissues indicates that the protein product of this gene is useful as a growth factor or differentiation factor for particular cell types in the developing fetus and may be useful in replacement or other types of therapy in cases where the gene is expressed aberrantly. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or

15 treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of

20 this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may

25 serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of

30 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

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In specific embodiments, polypeptides of the invention comprise, or

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MRAASPPASASDLIEQQQKGRREHKALIKQDNLD AFNERDPYKADD SRE  
(SEQ ID NO:493), EEEENDDDNSLEGETFPLERDEVMPPLQHPQTDR LX  
CPKGLPWX (SEQ ID NO:494), PKVREKDIEMFLESSRSKFIGYTLGSDTNTV  
VGLPRPIHESI KTLKQHKYT (SEQ ID NO:495), SIAEVQAQMEEEEYLR SPLSGG  
EEVEVQVPAETLYQG LPSLPQYMIA (SEQ ID NO:496), LLKILLAAPTSKAK  
TDSINILADVLP EEMPTTVLQSMKLGVDVNRHK (SEQ ID NO:497), EVIVKA

ISAVLLLLLKHFKLNHVYQFEYMAQHLVFANCIPLILKFFNQNI (SEQ ID NO:498),

MSYITAKNSISVLDYPHCVVHELPELTAESLEAGDSNQFCWRNLFSCI (SEQ ID NO:499), NLLRILNKLTKWKHSRTMMLVVFKSAPILKRALKVKQ

5 AMMQLYVLKL (SEQ ID NO:500),

LKVQTKYLGRQWRKSNMKTMSAIYQKVRH RLNDDDWAYGNDLDARP (SEQ ID NO:501), WDFQAEECALRANIERFNARRYDR

AHSNPDFLPVDNCLQSVLGQRVDL (SEQ ID NO:502), and

PEDFQMNYDLWLE REV FSKPISWEELLQ (SEQ ID NO:503). Moreover,

10 fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
15 of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The translation product of this gene shares sequence homology with a *C. elegans* protein (gi|1086830 coded for by *C. elegans* cDNA yk20f8.5).

20 This gene is expressed primarily in T-cells, and to a lesser extent in tumor tissue including glioblastoma, menangioma, and Wilm's tumor.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the immune system, including autoimmune conditions such as rheumatoid  
25 arthritis, inflammatory disorders and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or  
30 cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 260 as residues: Thr-9 to Ser-14.

5 The tissue distribution in T-cells indicates that the protein product of this gene is useful for the diagnosis and/or modulation of immune function disorders, including rheumatoid arthritis and inflammatory responses. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by  
10 boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
20 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1388 of SEQ ID NO:22, b is an integer of 15 to 1402, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 13

25 This gene is expressed primarily in placenta, and to a lesser extent in fetal liver and bone marrow.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample



and for the diagnosis of hematological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematological and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. placental, immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal liver, and bone marrow indicates that the protein product of this gene is useful as a growth factor for hematopoietic stem cells or progenitor cells in the treatment of chemotherapy patients or kidney disease. Furthermore, the tissue distribution in placenta indicates that the protein product of this gene is useful for the diagnosis and/or treatment of vascular or reproductive disorders. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1033 of SEQ ID NO:23, b is an integer of 15 to 1047, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 14

This gene is expressed primarily in stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of hematopoietic disorders including cancer, neutropenia, anemia, and thrombocytopenia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in stromal cells indicates that the protein product of this gene is useful as a growth factor for hematopoietic stem cells or progenitor cells, in particular following chemotherapy treatment. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in

lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 976 of SEQ ID NO:24, b is an integer of 15 to 990, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 15

The translation product of this gene shares sequence homology with epsilon-COP from *Bos taurus*, which is thought to be important as a component of coatomer, a complex of seven proteins, that is the major component of the non-clathrin membrane coat.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MAPPAPGPASGGSGEVDELFDVKNAFYIGSYQQCINEAXXVKLSSPERDVER  
 DVFLYRAYLAQRKFGVVLDEIKPSSAPELQAVRMFADYLAHESRRDSIVAEL  
 DREMSRSXDVTNTTFLMAASIYLHDQNPDAALRALHQGDSLECTAMTVQIL  
 LKLDRLDLARKELKRMQDLDEDATLTQLATAWVSLATGGEKLQDAYYIFQE  
 MADKCSPTLLLLNGQAACHMAQGRWEAAEGLLQEALDKDSGYPETLVNLIV  
 LSQHLGKPPEVTNRYLSQLKDAHRSHPIKEYQAKENDFDRLVLQYAPSAEA  
 GPELSGP (SEQ ID NO:504),

- RDVERDVFLYRAYLAQRKFGVVLDEIKPSSAPELQAVRMFADYLAHESRRDS  
IVAELDREMSRSXDVTNTTFLDMAASIYLHDQNPDAALRALHQGDSLECTAM  
TVQILLKLDRLDLARKELKRMQDLDEDATLTQLATAWVSLATGGEKLQDAY  
YIFQEMADKCSPTLLLLNGQAACHMAQGRWEAAEGLLQEALDKDSGYPETL  
5 VNLIVLSQHLGKPPEVTNRYLSQLKDAHRSHPIKEYQAKENDFDRLVLQYA  
PSA (SEQ ID NO:505),  
MAPPAPGPASGGSGEVDELFDVKNAFYIGSYQQCINEAXXVKLSSPER (SEQ  
ID NO:506),  
DVERDVFLYRAYLAQRKFGVVLDEIKPSSAPELQAVRMFADYLAHES (SEQ  
10 ID NO:507),  
RRDSIVAELDREMSRSXDVTNTTFLDMAASIYLHDQNPDAALRALHQG (SEQ  
ID NO:508),  
DSLECTAMTVQILLKLDRLDLARKELKRMQDLDEDATLTQLATAWVS (SEQ  
ID NO:509),  
15 LATGGEKLQDAYYIFQEMADKCSPTLLLLNGQAACHMAQGRWEAAEG  
(SEQ ID NO:510),  
LLQEALDKDSGYPETLVNLIVLSQHLGKPPEVTNRYLSQLKDAHRSHPI (SEQ  
ID NO:511), FIKEYQAKENDFDRLVLQYAPSAEAGPELSGP (SEQ ID NO:512),  
RDVERDVFLYRAYLAQRKFGVVLDEIKPSSAPELQAVRMFADYLAHE (SEQ  
20 ID NO:513),  
SRRDSIVAELDREMSRSXDVTNTTFLDMAASIYLHDQNPDAALRALHQ (SEQ  
ID NO:514),  
GDSLECTAMTVQILLKLDRLDLARKELKRMQDLDEDATLTQLATAWV (SEQ  
ID NO:515),  
25 SLATGGEKLQDAYYIFQEMADKCSPTLLLLNGQAACHMAQGRWEAAE (SEQ  
ID NO:516), GLLQEALDKDSGYPETLVNLIVLSQHLGKPPEVTNRYL (SEQ ID  
NO:517), SQLKDAHRSHPIKEYQAKENDFDRLVLQYAPSA (SEQ ID NO:518),  
or  
NRYRESWSLQVPVRNSGSTHASERNGASGPRPGLRRLRGGRRAVRRKERL  
30 LHRQLPAVHKR (SEQ ID NO:519). Moreover, fragments and variants of these  
polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides

and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 19. Accordingly, polynucleotides of the invention are useful as a marker in linkage analysis for chromosome 19.

This gene is expressed primarily in activated monocytes and T-cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunomodulation, specifically relating to transport problems in these cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in activated monocytes and T-cells combined with the homology to epsilon-COP indicates that the protein product of this gene is useful for treating and/or diagnosing problems with the cellular transport of proteins that may result in immunologic dysfunction. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1194 of SEQ ID NO:25, b is an integer of 15 to 1208, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 16**

The translation product of this gene shares sequence homology with an RNA helicase which is thought to be important in polynucleotide metabolism. The translation product of this contig exhibits good homology to the LbeIF4A antigen of *Leishmania braziliensis*. The LbeIF4A antigen, or immunogenic portions of it, can be used to induce protective immunity against leishmaniasis, specifically *L. donovani*, *L. chagasi*, *L. infantum*, *L. major*, *L. braziliensis*, *L. panamensis*, *L. tropica* and *L. guyanensis*. It can also be used diagnostically to detect *Leishmania* infection or to stimulate a cellular and/or humoral immune response or to stimulate the production of interleukin-12. The gene encoding the disclosed cDNA is thought to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in colon cancer, and to a lesser extent, in pituitary.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of cancers particularly of the colon. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of

disorders of the above tissues or cells, particularly of the gastrointestinal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. colon, pituitary, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 264 as residues: Glu-93 to Ala-98, Gln-150 to Leu-156, Leu-220 to Leu-231, Leu-268 to Arg-273, Val-324 to Pro-341, Arg-372 to Asn-380, Ser-405 to Gly-410, Phe-426 to Ala-433, Glu-458 to Asp-470, and/or Arg-506 to Ser-547.

The tissue distribution in colon cancer, combined with the homology to RNA helicase indicates that the protein product of this gene is useful for the development of diagnostic tests for colon cancer or other gastrointestinal or metabolic disorders.

Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1908 of SEQ ID NO:26, b is an integer of 15 to 1922, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 17

The translation product of this contig has sequence homology to a cytoplasmic protein that binds specifically to JNK, designated the JNK interacting protein-1 or JIP-1 in *Mus musculus*. JIP-1 caused cytoplasmic retention of JNK and inhibition of JNK-regulated gene expression. The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

APGXGWRGSLGEPPPPRASLSSDTSALSYSVKYTLVVDEHAQLELV

10 SLRRASETTVTRVTLPPS (SEQ ID NO:520),

APGXGWRGSLGEPPPPRASLSSDTSALSY (SEQ ID NO:521), or

DSVKYTLVVDEHAQLELVSLRRASETTVTRVTLPPS (SEQ ID NO:522).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,

15 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

20 This gene is expressed primarily in brain, including pituitary, cerebellum, frontal cortex, and fetal brain, and to a lesser extent in the cortex or the kidney.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the central nervous system disorders including ischemia,

25 epilepsy, Parkinson's disease, and schizophrenia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, kidney, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a

30



disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Furthermore, the translation product of this contig may suppress the effects of the JNK signaling pathway on cellular proliferation, including transformation by the Bcr-Abl oncogene.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 265 as residues: Pro-6 to Ser-26, Ala-30 to Asp-41, Gly-55 to Ser-61, Gly-74 to Thr-80, Tyr-117 to Ala-123, Tyr-167 to Asp-172, Ala-212 to Cys-223, and/or Pro-239 to Tyr-244.

The tissue distribution in brain indicates that the protein product of this gene is useful for the enhanced survival and/or differentiation of neurons as a treatment for neurodegenerative disease. Furthermore, the tissue distribution indicates that the translation product of this gene may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1937 of SEQ ID NO:27, b is an integer of 15 to 1951, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

The translation product of this gene shares sequence homology with a liver stage antigen from a protozoan parasite.

This gene is expressed primarily in fetal tissue, and to a lesser extent, in activated T-cells and other immune cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental abnormalities and diseases of immune function. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in T-cells, combined with the homology to a protozoan antigen indicates that the protein product of this gene is useful for the treatment and/or immune modulation of parasitic infections. Furthermore, expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3975 of SEQ ID NO:28, b is an integer of 15 to 3989, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 19

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 5 MKAIGIEPSLATYHHIIRLFDQPGDPLKRSSFIIYDIMNELMGKRFSPKDPDDD  
KFFQSAMSICSSLRDLELAYQVHGLLKTGDNWKFIPDQHRNFYYSKFFDLIC  
LMEQIDVTLKWYEDLIPSAYFPHSQTMIHLLQALDVANRLEVIPKIWER (SEQ  
ID NO:523),  
KDSKEYGHTFRSDLREEILMLMARDKHPPQLQVAFADCAADIKSAYESQPIRQ
  - 10 TAQDWPATSLNCIAILFLRAGRTQEAWKMLGLFRKHNKIPRSELLNELMDSA  
KVSNSPSQAIEVVELASAFSLPICEGLTQRVMSDFAINQEKEALSNLTALTSD  
SDTDSSSDSDSDTSEGK (SEQ ID NO:524),  
MKAIGIEPSLATYHHIIRLFDQPGDPLKRSSFIIYDIMNELMGKRFSPK (SEQ ID  
NO:525),
  - 15 DPDDDKFFQSAMSICSSLRDLELAYQVHGLLKTGDNWKFIPDQHRNFY  
(SEQ ID NO:526), YSKFFDLICLMEQIDVTLKWYEDLIPSA (SEQ ID NO:527),  
YFPHSQTMIHLLQALDVANRLEVIPKIWER (SEQ ID NO:528),  
KDSKEYGHTFRSDLREEILMLMARDKHPPQLQVAFADCAADIKSAY (SEQ ID  
NO:529),
  - 20 ESQPIRQTAQDWPATSLNCIAILFLRAGRTQEAWKMLGLFRKHNKIPRSE  
(SEQ ID NO:530),  
LLNELMDSAKVSNSPSQAIEVVELASAFSLPICEGLTQRVMSDFAIN (SEQ ID  
NO:531), or QEKEALSNLTALTSDSDTDSSSDSDSDTSEGK (SEQ ID NO:532).
- Moreover, fragments and variants of these polypeptides (such as, for example,
- 25 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides ) are encompassed by the invention. Antibodies that  
bind polypeptides of the invention are also encompassed by the invention.
  - 30 Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in stromal and CD34 depleted bone marrow cells, and to a lesser extent in tissues of embryonic origin.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of hematopoietic origin including cancers and immune dysfunction.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 267 as residues: Ser-28 to Gln-34.

The tissue distribution in stromal and CD34 depleted bone marrow cells indicates that the protein product of this gene is useful as a growth factor for hematopoietic stem cells or progenitor cells which may be useful in the treatment of chemotherapy patients suffering from neutropenia. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection,

inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3721 of SEQ ID NO:29, b is an integer of 15 to 3735, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 20

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MSSDNESDIEDEDLKLELRRLRDKHLKEIQDLQSRQKHEIESLYTKLGKVPPA  
VIIPPAAPLSGRRRRPTKSKGSKSSRSSSLGNKSPQLSGNLSGQSAASVLHPQQ  
TLHPPGNIPESGQNQLLQPLKPSPPSSDNL YSAFTSDGAISVPSLSAPGQGTSSSTN  
TVGATVNSQAAQAQPPAMTSSRKGTFTDDLHKLVDNWARDAMNLSGRRGS  
KGHMNYEGPGMARKFSAPGQLCISMTSNLGGAPISAASATSLGHFTKSMCP  
PQQYGFPATPFGAQWSGTGGPAPQPLGQFQPVGTASLQNFNISNLQKSISNPP  
GSNLRTT (SEQ ID NO:533),

IQDLQSRQKHEIESLYTKLGKVPPAVIIPPAAPLSGRRRRPTKSKGSKSSRSSSL  
GNKSPQLSGNLSGQSAASVLHPQQTLHPPGNIPESGQNQLLQPLKPSPPSSDNL  
YSAFTSDGAISVPSLSAPGQGT SST (SEQ ID NO:534),  
TSDGAISVPSLSAPGQGTSSSTNTVGATVNSQAAQAQPPAMTSSRKGTFTDDL  
H (SEQ ID NO:535),

KGHMNYEGPGMARKFSAPGQLCISMTSNLGGAPISAASATSLGHFTK (SEQ ID NO:536), QPLKPS SSDNL YSAFTSDGAISVPSLSAPG (SEQ ID NO:537), MSSDNESDIEDLDKLELRRLRD KHLKEIQDLQSRQKHEIESLYTKLGKVP (SEQ ID NO:538),

5 PAVIIPPAAPLSGRRRRPTKSKGSKSSRSSSLGNKSPQLSGNL SGQS (SEQ ID NO:539),

AASVLHPQQT LHPPGNIPESGQNQLLQPLKPS SSDNL YSAFTSDGAISV (SEQ ID NO:540), PLSAPGQGT SSTNTVGATVNSQAAQAQPPAMTSSRKGTFTDDL (SEQ ID NO:541),

10 HKLVDNWARDAMNLSGRRGSKGHMNYEGPGMARKFSAPGQLCISMT (SEQ ID NO:542),

SNLGGAPISAASATSLGHFTKSMCPPQQYGFPATPFGAQWSGTGG (SEQ ID NO:543), and PAPQPLGQFQPVGTASLQNFNISNLQKSISNPPGSNLRTT (SEQ ID NO:544). Moreover, fragments and variants of these polypeptides (such as, for

15 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

20 Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in fetal liver and tissues associated with the CNS.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,

25 liver and CNS diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver and CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell

30 types (e.g. liver, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression

level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 268 as residues: Gln-26 to Lys-34.

5       The tissue distribution in fetal liver and neural tissues indicates that the protein product of this gene is useful for the diagnosis and treatment for liver diseases such as hepatocellular carcinomas and diseases of the CNS. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells), as well as the detection and treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception.

15       Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1653 of SEQ ID NO:30, b is an integer of 15 to 1667, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 21**

30       The translation product of this gene shows sequence homology to two recently cloned genes, karyopherin beta 3 and Ran\_GTP binding protein 5. (See Genbank

Accession Nos. gi|2102696 and gnl|PID|e328731.) The Ran\_GTP binding protein is related to importin-beta, the key mediator of nuclear localization signal (NLS)-dependent nuclear transport. Based on homology, it is likely that this gene may demonstrate activity similar to the RAN\_GTP binding protein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

VRVAAAESMXLLLECA XVRGPEYLTQMWHFMCDALIK AIGTEPDSDVLSEI  
MHSFAK (SEQ ID NO:545). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed in thymus tissue, and to a lesser extent in stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, thymus, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in thymus indicates that the protein product of this gene is useful for the diagnosis and treatment for immune disorders. Furthermore, the polypeptides or polynucleotides of the present invention are also useful in the



treatment, prophylaxis, and detection of thymus disorders, such as Graves Disease, lymphocytic thyroiditis, hyperthyroidism, and hypothyroidism. Additionally, the tissue distribution indicates that the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia,

5 pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such  
10 as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Many polynucleotide sequences, such as EST sequences, are publicly  
15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
20 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1394 of SEQ ID NO:31, b is an integer of 15 to 1408, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 22**

The translation product of this gene shares sequence homology with a natural resistance-associated macrophage protein 2 from Homo sapiens (gi|3152690  
30 (AF064484)), which is thought to function as a macrophage-specific membrane transport protein. This gene is expressed primarily in prostate and osteoclastoma tissues. In specific embodiments, polypeptides of the invention comprise, or

alternatively consists of, an amino acid sequence selected from the group:

MEINNQNCFIVIDLVRTVMENGVEGLLIFGAFLPESWLIGVRCSSSEPPKALLLIL  
AHSQKRRLDGWFSIRHLRVHYCVSLTIHFS (SEQ ID NO:546),

GGREANKXFFIESCIALFVSFIINVSVFAEXFFGXTNEQVVEVCTNTSSPH

5 AGLFPKDNSTLAVDIYKGGVVLGCYFGPAALYIWAVGILAAGQSST (SEQ ID  
NO:547), GGREANKXFFIESCIALFVSFIINVSVFAEXFFGXTNEQVVE  
(SEQ ID NO:548), and/or

VCTNTSSPHAGLFPKDNSTLAVDIYKGGVVLGCYFGPAALYIWAVGILAAGQ  
SST (SEQ ID NO:549). Moreover, fragments and variants of these polypeptides

10 (such as, for example, fragments as described herein, polypeptides at least 80%, 85%,  
90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides ) are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the  
15 invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome  
12. Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 12.

20 This gene is expressed primarily in fetal liver/spleen, fetal brain, and to a  
lesser extent in placenta.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
25 immune, developmental, hepatic, or bone and prostate diseases, and cancers,  
particularly of the bone and prostate. Similarly, polypeptides and antibodies directed  
to these polypeptides are useful in providing immunological probes for differential  
identification of the tissue(s) or cell type(s). For a number of disorders of the above  
tissues or cells, particularly of the bone and prostate systems, expression of this gene  
30 at significantly higher or lower levels may be routinely detected in certain tissues or  
cell types (e.g. bone, prostate, cancerous and wounded tissues) or bodily fluids (e.g.  
lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell

sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in bone indicates that the protein product of this gene is useful for the diagnosis and treatment of bone and prostate disorders, especially cancers of those systems. Elevated levels of expression of this gene product in osteoclastoma indicates that it may play a role in the survival, proliferation, and/or growth of osteoclasts. Therefore, it may be useful in influencing bone mass in such conditions as osteoporosis. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3172 of SEQ ID NO:32, b is an integer of 15 to 3186, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 23

This gene shares sequence homology with the FK506-binding protein (FKBP-13) family, a known cytosolic receptor for the immunosuppressants FK506 and rapamycin. Recently, another group has cloned a very similar gene, recognizing the homology to the FK506-binding protein family, calling their gene FKBP23 (See Genbank Accession No. 2827255.). Contact of cells with supernatant expressing the product of this gene increases the permeability of both prostate stromal cells and dermal fibroblasts to calcium. Thus, it is likely that the product of this gene is involved in a signal transduction pathway that is initiated when the product of this gene binds receptors on the surface of stromal cells and dermal fibroblast cells. Thus, polynucleotides and polypeptides have uses which include, but are not limited to, activating stromal and fibroblast cells.

This gene is expressed primarily in lymphoid tissues and stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample, especially for those susceptible to immune suppressant therapies and for diagnosis of diseases and conditions which include, but are not limited to, immune suppressant disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 271 as residues: Ala-19 to Val-31, Arg-38 to Gly-49, Ala-61 to Lys-66, Tyr-68

to Pro-78, Gly-116 to Ala-121, Asp-154 to Ser-162, Glu-173 to Gln-186, Phe-194 to Gly-203, and/or Pro-207 to Val-212.

The tissue distribution in lymphoid tissues and stromal cells, the biological activity data, combined with the homology to FKBP-12 and -13 indicates that the protein product of this gene is useful for the diagnosis and treatment of immune suppressant disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 957 of SEQ ID NO:33, b is an integer of 15 to 971, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 24**

The gene encoding the disclosed cDNA is thought to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

This gene is expressed primarily in the brain and in the retina.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological and ocular associated disease states. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the disorders of the central nervous system, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 272 as residues: Cys-34 to Asp-40.

10 The tissue distribution in retina indicates that the protein product of this gene is useful for the treatment and/or detection of eye disorders including blindness, color blindness, impaired vision, short and long sightedness, retinitis pigmentosa, retinitis proliferans, and retinoblastoma. Expression in the brain indicates a role in the is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1778 of SEQ ID NO:34, b is an integer of 15 to 1792, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 25**

30 This gene shows sequence homology to a newly identified class of proteins expressed in the nervous system, called stathmin family. (See Genbank Accession No. 2585991; see also Eur. J. Biochem. 248 (3), 794-806 (1997).) The stathmin

family appears to be an ubiquitous phosphoprotein involved as a relay integrating various intracellular signaling pathways. These pathways affect cell proliferation and differentiation.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

QDKHAEVVRKNKELKEEASR (SEQ ID NO:550),

QQDLSPWAAPVGCPLXXASXTCHXLPLSGCLRRQSXSLPVVAXLCFWFSCPL  
ASLFVPGQPCVTCFPLPFQDKHAEVVRKNKELKEEASR (SEQ ID NO:551).

Moreover, fragments and variants of these polypeptides (such as, for example,

fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed highly in brain tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease,

schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 882 of SEQ ID NO:35, b is an integer of 15 to 896, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 26**

The polynucleotide sequence of this gene contains a domain similar to a Flt3 ligand peptide.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

PTRCCTTQPCRSSARRPCWVPMVPSPEGREXQPTCPS (SEQ ID NO:552).

Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene may have activity as binding to Flt3 receptors, a process known to promote angiogenesis and/or lymphangiogenesis.

This gene is expressed in human tonsil, and to a lesser extent in teratocarcinoma, placenta, colon carcinoma, and fetal kidney.



Therefore, polynucleotides and polypeptides of the invention are useful as reagents for identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the tonsil, as well as cancers, such as colon, reproductive, and kidney cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the tonsils, colon, reproductive organs, and kidneys, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, tonsils, colon, kidney, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 274 as residues: Pro-22 to Glu-33.

The tissue distribution in tonsils, several cancers, and fetal tissues indicates that the protein product of this gene is useful for the diagnosis and treatment of diseases of the tonsil or colon, such as tonsillitis, inflammatory diseases involving nose and paranasal sinuses, especially during the infection of influenza, adenoviruses, parainfluenza, or rhinoviruses, for example. The gene may also be useful in the diagnosis and treatment of neoplasms of nasopharynx or colon origins. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 898 of SEQ ID NO:36, b is an

integer of 15 to 912, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 27

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

10 MKRSLNENSARSTAGCLPVPLFNQKKRNRQPLTSNPLKDDSGISTPSDNYDFP  
PLPTDWAWEAVNPEXAPVMKTVDTGQIPHSVSRPLRSQDSVFNSIQSNTGRS  
QGGWSYRDGNKNTSLKTWXKNDFKPQCKRTNLVANDGKNSCPMSSGAQQ  
QKQLRTPEPPNLSRNKETELLRQTHSSKISGCTMRGLDKNSALQTLKPNFQQN  
QYKXQMLDDIPEDNTLKETS LYQLQFKEKASSLRISAVIESMKYWREHAQKT  
VLLFEVLAVLDSAVTPGPYYSKTFLMRDGKNTLPCVFYEIDRELPRILIRGRVH

15 RCVGN YDQKKNIFQCVSVRPASVSEQKTFQAFVKIADVEMQYYINVMNET  
(SEQ ID NO:553),

SQDSVFNSIQSNTGRSQGGWSYRDGNKNTSLKTWXKNDFKPQCKR (SEQ ID  
NO:554), NKETELLRQTHSSKISGCTMRGLDKNSALQTLKPNF (SEQ ID  
NO:555),

20 SSLRIISAVIESMKYWREHAQKTVLLFEVLAVLDSAVTPGPYYSKTFLM (SEQ  
ID NO:556), and/or

PRLIRGRVHRCVGN YDQKKNIFQCVSVRPASVSEQKTFQAFV (SEQ ID  
NO:557). Moreover, fragments and variants of these polypeptides (such as, for

25 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides ) are encompassed by the invention. Antibodies that  
bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

30 This gene is expressed primarily in human testes.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include, but are not limited to, male reproductive disorders, including cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. testes, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human testes indicates that the protein product of this gene is useful as a hormone with reproductive or other systemic functions; contraceptive development; male infertility of testicular causes, such as Klinefelter's syndrome, varicocele, orchitis; male sexual dysfunctions; testicular neoplasms; and inflammatory disorders such as epididymitis. Furthermore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

formula of a-b, where a is any integer between 1 to 1368 of SEQ ID NO:37, b is an integer of 15 to 1382, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

5

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 28**

This gene is expressed primarily in apoptotic T-cell.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases relating to T cells, as well as cancer in general. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the disorders of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in apoptotic T-cells indicates that the protein product of this gene is useful for the detection and/or treatment of disorders of the immune system. Moreover, since the gene was isolated from an apoptotic cell, and based on the understanding of the relationship of apoptosis and cancer, it is likely that this gene may play a role in the genesis of cancer. Furthermore, expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 858 of SEQ ID NO:38, b is an integer of 15 to 872, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 29

This gene is expressed primarily in human tonsils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, gastrointestinal and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human tonsils indicates that the protein product of this gene is useful for the diagnosis and treatment of gastrointestinal diseases. Alternatively, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and treatment of a variety of immune system disorders. Expression of this gene product in tonsils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other

processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or  
10 proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
15 related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
20 formula of a-b, where a is any integer between 1 to 798 of SEQ ID NO:39, b is an integer of 15 to 812, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO:30**

25 This gene is expressed primarily in human T-cells, and to a lesser extent, in human colon carcinoma.

The translation product of this gene shares sequence homology with C44C1.2 gene product of *Caenorhabditis elegans*.

Preferred polypeptides of the present invention comprise, or alternatively  
30 consist of, one, two, three, four, five, six, seven or all seven of the immunogenic epitopes shown in SEQ ID NO:278 as residues: Leu-21 to Ala-30, Ser-38 to Asp-47, Pro-87 to Asp-94, Leu-197 to Thr-204, Pro-256 to Ser-262, Thr-277 to Arg-282,

and/or Thr-293 to Trp-303. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these peptides.

Additionally, preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, or both of the immunogenic epitopes shown in SEQ ID NO:1232 as residues: Gly-204 to Gly-234 and Arg-202 to Asp-236.

Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides.

In additional nonexclusive embodiments, preferred polypeptides of the invention also comprise, or alternatively consist of, one or more of the following amino acid sequences: Gly-188 to Val-203, Gly-188 to Thr-204, Thr-204 to Lys-257, Asp-280 to Leu-362 of SEQ ID 278 and Gly-204 to Gly-234 of SEQ ID NO: 1232. Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these peptides.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

GVFRPCVCGRPASLTCSPLDPEVGPYCDTPTMRTLFNLLWLALACSPVHTTLS  
KSDAKKAASKTLLEKSQFSDKPVQDRGLVVTDLKAESVVLEHRSYCSAKAR  
DRHFAGDVLGYVTPWNSHGYDVTKVFGSKFTQISPVWLQLKRRGREMFEVT  
GLHDVDQGWMRAVRKHAKGLHIVPRLLFEDWTYDDFRNVLDSEDEIEELSK  
TVVQVAKNQHFDFGVVEVWNQLLSQKRVGLIHMLTHLAEALHQARLLALL  
VIPPAITPGTDQLGMFTHKEFEQLAPVLDGFSLMTYDYSTAHQPGPNAPLSWV  
RACVQVLDPKXKWRTKSSWGSTSMXWTRXPXDARXPVVGXRQIXLKD  
HXPVMVLDSPKQ (SEQ ID NO:558),

TCSPLDPEVGPYCDTPTMRTLFNLLWLALACSPVHTTLS (SEQ ID NO:559),  
LVVTDLKAESVVLEHRSYCSAKARDRHAGDVLGYVTPWNSHGYDVTKVF  
GSKF (SEQ ID NO:560),  
REMFEVTGLHDVDQGWMRAVRKHAKGLHIVPRLLFEDWTYDDFRNVLDSE  
DE (SEQ ID NO:561),

HFDGFVVEVWNQLLSQKRVGLIHMLTHLAEALHQARLLALLVIPPAITPGTD  
QLGM (SEQ ID NO:562), and  
DGFSLMTYDYSTAHQPGPNAPLSWVRACVQVLDPKXKWRTKSSWGST (SEQ

ID NO:563). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In additional nonexclusive embodiments, polynucleotides of the invention comprise or alternatively consist of, one or more of the following sequences:

10 GGCACGAGCGTTTTCCGGCCGTGCGTTTTGTGGCCGTCCGGCCTCCC  
 TGACATGCAGCCCTCTGGACCCCGAGGTTGGACCCTACTGTGACACACCT  
 ACCATGCGGACACTCTTCAACCTCCTCTGGCTTGCCCTGGCCTGCAGCCCT  
 GTTCACACTACCCTGTCAAAGTCAGATGCCAAAAAGCCGCCTCAAAGAC  
 GCTGCTGGAGAAGAGTCAGTTTTTCAGATAAGCCGGTGCAAGACCGGGGTT  
 15 TGGTGGTGACGGACCTCAAAGCTGAGAGTGTGGTTCTTGAGCATCGCAGC  
 TACTGCTCGGCAAAGGCCCGGGACAGACACTTTGCTGGGGATGTACTGGG  
 CTATGTCACTCCATGGAACAGCCATGGCTACGATGTCACCAAGGTCTTTG  
 GGAGCAAGTTCACACAGATCTCACCCGTCTGGCTGCAGCTGAAGAGACGT  
 GGCCGTGAGATGTTTGAGGTCACGGGCCTCCACGACGTGGACCAAGGGTG  
 20 GATGCGAGCTGTCAGGAAGCATGCCAAGGGCCTGCACATAGTGCCTCGGC  
 TCCTGTTTGAGGACTGGACTTACGATGATTTCCGGAACGTCTTAGACAGTG  
 AGGATGAGATAGAGGAGCTGAGCAAGACCGTGGTCCAGGTGGCAAAGAA  
 CCAGCATTTTCGATGGCTTCGTGGTGGAGGTCTGGAACCAGCTGCTAAGCC  
 AGAAGCGCGTGCGGCCTCATCCACATGCTCACCCACTTGGCCGAGGCTCTG  
 25 CACCAGGCCCGGCTGCTGGCCCTCCTGGTCATCCCGCCTGCCATCACCCCC  
 GGGACCGACCAGCTGGGCATGTTACGCACAAGGAGTTTGAGCAGCTGGC  
 CCGCGTGCTGGATGGTTTCAGCCTCATGACCTACGACTACTCTACAGCGCA  
 TCAGCCTGGCCCTAATGCACCCCTGTCCTGGGTTCGAGCCTGCGTCCAGGT  
 CCTGGACCCGAAGTCCAAGTGGCGAAGCAAAATCCTCCTGGGGCTCAACT  
 30 TCTATGGTACATCCAGACACTGAAGGACCACAGGCCCGGATGGTGTGGG  
 ACAGCCAGGTCTCAGAGCACTTCTTCGAGTACAAGAAGAGCCGCAGTGGG  
 AGGCACGTCGTCTTCTACCCAACCCTGAAGTCCCTGCAGGTGCGGCTGGA



GCTGGCCCCGGGAGCTGGGCGTTGGGGTCTCTATCTGGGAGCTGGGCCAGG  
 GCCTGGACTACTTCTACGACCTGCTCTAGGTGGGCATTGCGGCCTCCGCGG  
 TGGACGTGTTCTTTTCTAAGCCATGGAGTGAGTGAGCAGGTGTGAAATAC  
 AGGCCTCCACTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
 5 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:1228),  
 GCGCTGGAGCGTTTTCCGGCCGTGCGTTTGTGGCCGTCCGGCCTCCCTGAC  
 ATGCAGCCCTCTGGACCCCGAGGTTGGACCCTACTGTGACACACCTACCA  
 TGCGGACACTCTTCAACCTCCTCTGGCTTGCCCTGGCCTGCAGCCCTGTTC  
 ACACTACCCTGTCAAAGTCAGATGCCAAAAAGCCGCCTCAAAGACGCTG  
 10 CTGGAGAAGAGTCAGTTTTTCAGATAAGCCGGTGCAAGACCGGGGTTTGGT  
 GGTGACGGACCTCAAAGCTGAGAGTGTGGTTCTTGAGCATCGCAGCTACT  
 GCTCGGCAAAGGCCCGGGACAGACACTTTGCTGGGGATGTACTGGGCTAT  
 GTCACTCCATGGAACAGCCATGGCTACGATGTCACCAAGGTCTTTGGGAG  
 CAAGTTCACACAGATCTCACCCGTCTGGCTGCAGCTGAAGAGACGTGGCC  
 15 GTGAGATGTTTGAGGTCACGGGCCTCCACGACGTGGACCAAGGGTGGATG  
 CGAGCTGTCAGGAAGCATGCCAAGGGCCTGCACATAGTGCCTCGGCTCCT  
 GTTTGAGGACTGGACTTACGATGATTTCCGGAACGTCTTAGACAGTGAGG  
 ATGAGATAGAGGAGCTGAGCAAGACCGTGGTCCAGGTGGCAAAGAACCA  
 GCATTTTCGATGGCTTCGTGGTGGAGGTCTGGAACCAGCTGCTAAGCCAGA  
 20 AGCGCGTGACCGACCAGCTGGGCATGTTACGCACAAGGAGTTTGAGCAG  
 CTGGCCCCCGTGCTGGATGGTTTCAGCCTCATGACCTACGACTACTCTACA  
 GCGCATCAGCCTGGCCCTAATGCACCCCTGTCTGGGTTCGAGCCTGCGTC  
 CAGGTCCTGGACCCGAAGTCCAAGTGGCGAAGCAAAATCCTCCTGGGGCT  
 CAACTTCTATGGTATGGACTACGCGACCTCCAAGGATGCCCGTGAGCCTG  
 25 TTGTCGGGGCCAGGTACATCCAGACACTGAAGGACCACAGGCCCGGATG  
 GTGTGGGACAGCCAGGYCTCAGAGCACTTCTTCGAGTACAAGAAGAGCCG  
 CAGTGGGAGGCACGTCGTCTTCTACCCAACCCTGAAGTCCCTGCAGGTGC  
 GGCTGGAGCTGGCCCCGGGAGCTGGGCGTTGGGGTCTCTATCTGGGAGCTG  
 GGCCAGGGCCTGGACTACTTCTACGACCTGCTCTAGGTGGGCATTGCGGC  
 30 CTCCGCGGTGGACGTGTTCTTTTCTAAGCCATGGAGTGAGTGAGCAGGTG  
 TGAAATACAGGCCTNCACTCCGTTCAAAAAAAAAAAAAAAAAAAAAAA  
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACTCGAG (SEQ ID NO: 1229),

GGCGTTTTCCGGCCGTGCGTTTGTGGCCGTCCGGCCTCCCTGACATGCAGC  
 CCTCTGGACCCCGAGGTTGGACCCTACTGTGACACACCTACCATGCGGAC  
 ACTCTTCAACCTCCTCTGGCTTGCCCTGGCCTGCAGCCCTGTTCACTAC  
 CCTGTCAAAGTCAGATGCCAAAAAAGCCGCCTCAAAGACGCTGCTGGAGA  
 5 AGAGTCAGTTTTTCAGATAAGCCGGTGCAAGACCGGGGTTTGGTGGTGACG  
 GACCTCAAAGCTGAGAGTGTGGTTCTTGAGCATCGCAGCTaCTGCTcGGCA  
 AAGGCCCGGGACAGACACTTTGCTGGGGATGTACTGGGCTATGTCACTCC  
 ATGGAACAGCCATGGCTACGATGTCACCAAGGTCTTTGGGAGCAAGTTCA  
 CACAGATCTCACCCGTCTGGCTGCAGCTGAAGAGACGTGGCCGTGAGATG  
 10 TTTGAGGTCACGGGCCTCCACGACGTGGACCAAGGGTGGATGCGAGCTGT  
 CAGGAAGCATGCCAAGGGCCTGCACATAGTGCCTCGGCTCCTGTTTGAGG  
 ACTGGACTTACGATGATTTCCGGAACGTCTTAGACAGTGAGGATGAGATA  
 GAGGAGCTGAGCAAGACCGTGGTCCAGGTGGCAAAGAACCAGCATTTCG  
 ATGGCTTCGTGGTGGAGGTCTGGAACCAGCTGCTAAGCCAGAAGCGCGTG  
 15 GGCCTCATCCACATGCTCACCCACTTGGCCGAGGCTCTGCACCAGGCCCCG  
 GCTGCTGGCCCTCCTGGTCATCCCGCCTGCCATCACCCCGGGACCGACC  
 AGCTGGGCATGTTACGCACAAGGAGTTTGAGCAGCTGGCCCCCGTGCTG  
 GATGGTTTCAGCCTCATGACCTACGACTACTCTACAGCGCATCAGCCTGGc  
 CCTAATGCACCCcTGTCTGGGTTTCGAGCCTGCGTCCAGGTCCTGGACCCG  
 20 AARTYCAAGTGGCGAACAAAATCCTCCTGGGGSTCAACTTCTATGGWATG  
 GACTAMGCGACYTCCAANGGATGCCCCGTKARCCTGTTGTCGGGGSCAGGT  
 AMATYCAGAMACTGAARGACCACANGCCCCGGATGGTGTGGACAGCAA  
 GCCTCAAAG (SEQ ID NO:1230), and  
 ATAAGAGACAGCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAAC  
 25 GCGGNCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCT  
 TTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGT  
 GAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT  
 GAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCG  
 CGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAA  
 30 AGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGG  
 CACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTG  
 TGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCC

AAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCG  
 CGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTGCAGGAATTCTG  
 GCACGAGGTCCGGCCTCCCTGACATGCAGATTTCCACCCAGAAGACAGAG  
 AAGGAGCCAGTGGTCATGGAATGGGCTGGGGTCAAAGACTGGGTGCCTG  
 5 GGAGCTGAGGCAGCCACCGTTTCAGCCTGGCCAGCCCTCTGGACCCCGAG  
 GTTGGACCCTACTGTGACACACCTACCATGCGGACACTCTTCAACCTCCTC  
 TGGCTTGCCCTGGCCTGCAGCCCTGTTCACTACTACCCTGTCAAAGTCAGAT  
 GCCAAAAAAGCCGCCTCAAAGACGCTGCTGGAGAAGAGTCAGTTTTTCAGA  
 TAAGCCGGTGCAAGACCGGGGTTTGGTGGTGACGGACCTCAAAGCTGAGA  
 10 GTGTGGTTCTTGAGCATCGCAGCTACTGCTCGGCAAAGGCCCGGGACAGA  
 CACTTTGCTGGGGATGTACTGGGCTATGTCACTCCATGGAACAGCCATGG  
 CTACGATGTCACCAAGGTCTTTGGGAGCAAGTTCACACAGATCTCACCCG  
 TCTGGCTGCAGCTGAAGAGACGTGGCCGTGAGATGTTTGAGGTCACGGGC  
 CTCCACGACGTGGACCAAGGGTGGATGCGAGCTGTCAGGAAGCATGCCA  
 15 AGGGCCTGCACATAGTGCCTCGGCTCCTGTTTGAGGACTGGACTTACGAT  
 GATTTCCGGAACGTCTTAGACAGTGAGGATGAGATAGAGGAGCTGAGCA  
 AGACCGTGGTCCAGGTGGCAAAGAACCAGCATTTCGATGGCTTCGTGGTG  
 GAGGTCTGGAACCAGCTGCTAAGCCAGAAGCGCGTGGGCCTCATCCACAT  
 GCTCACCCACTTGGCCGAGGCTCTGCACCAGGCCCGGCTGCTGGCCCTCC  
 20 TGGTCATCCCGCCTGCCATCACCCCCGGGACCGACCAGCTGGGCATGTTC  
 ACGCACAAGGAGTTTGAGCAGCTGGCCCCCGTGCTGGATGGTTTCAGCCT  
 CATGACCTACGACTACTCTACAGCGCATCAGCCTGGCCCTAATGCACCCC  
 TGTCTGGGTTTCGAGCCTGCGTCCAGGTCCTGGACCCGAAGTCCAAGTGG  
 CGAAGCAAAATCCTCCTGGGGCTCAACTTCTATGGTACATCCAGACACTG  
 25 AAGGACCACAGGCCCCGGATGGTGTGGGACAGCCAGGCCTCAGAGCACT  
 TCTTCGAGTACAAGAAGAGCCGCAGTGGGAGGCACGTCGTCTTCTACCCA  
 ACCCTGAAGTCCCTGCAGGTGCGGCTGGAGCTGGCCCCGGGAGCTGGGCGT  
 TGGGGTCTCTATCTGGGAGCTGGGCCAGGGCCTGGACTACTTCTACGACC  
 TGCTCTAGGTGGGCATTGCGGCCTCCGCGGTGGACGTGTTCTTTTCTAAGC  
 30 CATGGAGTGAGTGAGCAGGTGTGAAATACAGGCCTCCACTCCGTAAAAA  
 AAAAAAAAAAAAAAAAAAACTCGAGGGGGGGCCCCGGTACCCAATTCGCCC  
 TATAGTGAGTCGTATTACAATTCCTGGCCGTGTTTTACAACGTCGTGAC

TGGGAAAACCCTGGCGTTACCCAACCTAATCGCCTTGCAGCACATCCCCCT  
 TTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCA  
 ACAGTTGCGCAGCCTGAATGGCGAATGGCAAATTGTAAGCGTTAATATTT  
 TGTTAAAATTTCGCGTTAAATTTTTTGTTAAATCAGCTCATTTTTTTAACCAAT  
 5 AGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGAT  
 AGGGTTGAGTGTTGNTCCAGTTTGGACAAGAGTCCACTATTAAAGAACG  
 TGGACTCCAACGTCAAAGGGCGAAAAACCGNCTATCAGGGCGATGGCCC  
 ACTACGTGAACCATCACCTTAATCAAAGTTTTTTGGGGTCGAGGTNCCCC  
 TAAAAGCACTTAATCGGGAACCC (SEQ ID NO:1231). Polypeptides encoded  
 10 by these polynucleotides are also encompassed by the invention, as are antibodies that  
 bind to these polypeptides.

In other specific embodiments, polypeptides of the invention comprise, or  
 alternatively consists of, an amino acid sequence selected from the group:

MRTLFLNLLWLALACSPVHTTSLKSDAKKAASKTLLEKSQFSDKPVQDRGLVV  
 15 TDLKAESVVLEHRSYCSAKARDRH FAGDVLGYVTPWNSHGYDVTKVFGSKF  
 TQISPVWLQLKRRGREMFVETGLHDVDQGWMRAVRKHAKGLHIVPRLLFED  
 WTYDDFRNVLDSEDEIEELSKTVVQVAKNQHFDFVVEVWNQLLSQKRVGL  
 IHMLTHLAEALHQARLLALLVIPAITPGTDQLGMFTHKEFEQLAPVLDGFSL  
 MTYDYSTAHPGPNAPLSWVRACVQVLDPKSKWRSKILLGLNFYGTSRH

(SEQ ID NO: 1232),

MRTLFLNLLWLALACSPVHTTSLKSDAKKAASKTLLEKSQFSDKPVQDRGLVV  
 TDLKAESVVLEHRSYCSAKARDRH FAGDVLGYVTPWNSHGYDVTKVFGSKF  
 TQISPVWLQLKRRGREMFVETGLHDVDQGWMRAVRKHAKGLHIVPRLLFED  
 WTYDDFRNVLDSEDEIEELSKTVVQVAKNQHFDFVVEVWNQLLSQKRVTD  
 25 QLGMFTHKEFEQLAPVLDGFSLMTYDYSTAHPGPNAPLSWVRACVQVLDP  
 KSKWRSKILLGLNFYGM DYATSKDAREPVVGARYIQTLKDHRPRMVWDSQ  
 XSEHFFEYKKSRSRGRHVVFYPTLKS LQVRLELARELGVGVS IWELGQGLDYF  
 YDLL (SEQ ID NO: 1233),

MRTLFLNLLWLALACSPVHTTSLKSDAKKAASKTLLEKSQFSDKPVQDRGLVV  
 30 TDLKAESVVLEHRSYCSAKARDRH FAGDVLGYVTPWNSHGYDVTKVFGSKF  
 TQISPVWLQLKRRGREMFVETGLHDVDQGWMRAVRKHAKGLHIVPRLLFED  
 WTYDDFRNVLDSEDEIEELSKTVVQVAKNQHFDFVVEVWNQLLSQKRVGL

IHMLTHLAEALHQARLLALLVIPPAITPGTDQLGMFTHKEFEQLAPVLDGFSL  
MTYDYSTAHPGP NAPLSWVRACVQVLDPKXKWRTKSSWGSTSMXWTRX  
XPXDARXPVVGXRX (SEQ ID NO: 1234), and

MRTLFNLLWLALACSPVHTTLSKSDAKKAASKTLLEKSQFSDKPVQDRGLVV  
5 TDLKAESVVLEHRSYCSAKARDRH FAGDVLGYVTPWNSHGYDVTKVFGSKF  
TQISPVWLQLKRRGREMFVETGLHDVDQGWMRVRKHAKGLHIVPRLLFED  
WTYDDFRNVLDSEDEIEELSKTVVQVAKNQHFDFVVEVWNQLLSQKRVGL  
IHMLTHLAEALHQARLLALLVIPPAITPGTDQLGMFTHKEFEQLAPVLDGFSL  
MTYDYSTAHPGP NAPLSWVRACVQVLDPKSKWRSKILLGLNFYGTSRH

10 (SEQ ID NO: 1235). Moreover, fragments and variants of these polypeptides (such  
as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides ) are encompassed by the invention.  
15 Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

Also preferred are polypeptides, comprising or alternatively consisting of, the  
mature polypeptide which is predicted to consist of residues 23-362 of the foregoing  
20 sequence (SEQ ID NO:278), and biologically active fragments of the mature  
polypeptide (e.g., fragments that inhibit the Mixed Lymphocyte Reaction).  
Polynucleotides encoding these polypeptides are also encompassed by the invention

Figures 1A-B show the nucleotide (SEQ ID NO:40) and deduced amino acid  
sequence (SEQ ID NO: 278) corresponding to this gene.

25 Figure 2 shows an analysis of the amino acid sequence (SEQ ID NO: 278).  
Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic  
regions; flexible regions; antigenic index and surface probability are shown, and all  
were generated using the default settings of the recited computer algorithms. In the  
"Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the  
30 highly antigenic regions of the protein, i.e., regions from which epitope-bearing  
peptides of the invention can be obtained. Polypeptides comprising, or alternatively

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consisting of, domains defined by these graphs are contemplated by the present invention, as are polynucleotides encoding these polypeptides.

The data presented in Figure 2 are also represented in tabular form in Table 3.

The columns are labeled with the headings "Res", "Position", and Roman Numerals

I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 2, and Table 3: "Res": amino acid residue of SEQ ID

NO: 278 and Figures 1A and 1B; "Position": position of the corresponding residue

within SEQ ID NO: 278 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson;

II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta,

Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions -

Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot -

Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic

Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible

Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface

Probability Plot - Emini.

Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consisting of, one or more of the following regions: alpha-

helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet

forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil

and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions,

alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-

forming regions and high antigenic index regions. The data representing the

structural or functional attributes of the protein set forth in Figure 2 and/or Table 3, as

described above, was generated using the various modules and algorithms of the

DNA\*STAR set on default parameters. In a preferred embodiment, the data

presented in columns VIII, IX, XIII, and XIV of Table 3 can be used to determine

regions of the protein which exhibit a high degree of potential for antigenicity.

Regions of high antigenicity are determined from the data presented in columns VIII,

IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide

which are likely to be exposed on the surface of the polypeptide in an environment in

which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figure 2, but may, as shown in Table 3, be represented or identified by using tabular representations of the data presented in Figure 2. The DNA\*STAR computer algorithm used to generate Figure 2 (set on the original default parameters) was used to present the data in Figure 2 in a tabular format (See Table 3). The tabular format of the data in Figure 2 is used to easily determine specific boundaries of a preferred region.

The present invention is further directed to fragments of the polynucleotide sequences described herein. By a fragment of, for example, the polynucleotide sequence of a deposited cDNA or the nucleotide sequence shown in SEQ ID NO:40, is intended polynucleotide fragments at least about 15nt, and more preferably at least about 20 nt, at least about 25nt, still more preferably at least about 30 nt, at least about 35nt, and even more preferably, at least about 40 nt in length, at least about 45nt in length, at least about 50nt in length, at least about 60nt in length, at least about 70nt in length, at least about 80nt in length, at least about 90nt in length, at least about 100nt in length, at least about 125nt in length, at least about 150nt in length, at least about 175nt in length, which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 200-1500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the nucleotide sequence of a deposited cDNA or as shown in SEQ ID NO:40. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:40. In this context "about" includes the particularly recited size, an sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Representative examples of polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to about 50, from about 51 to about 100, from about 101 to about 150, from about 151 to about 200, from about 201 to about 250, from about 251 to about 300, from about 301 to about 350, from about 351 to about 400, from about 401 to about 450, from about 451 to about 500, and from about 501 to about 550, and from about 551 to about 600, from about 601 to about 650, from about 651 to about 700, from about 701 to about 750, from about 751 to about 800, from about 801 to about 850, from about 851 to

about 900, from about 901 to about 950, from about 951 to about 1000, from about 1001 to about 1050, from about 1051 to about 1100, from about 1101 to about 1150 from about 1151 to about 1200, from about 1201 to about 1250, from about 1251 to about 1300, from about 1301 to about 1350, from about 1351 to about 1400, from about 1401 to about 1450, and from about 1451 to about 1515, of SEQ ID NO:40, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In additional embodiments, the polynucleotides of the invention encode functional attributes of the corresponding protein.

Preferred polypeptide fragments of the invention comprise, or alternatively consist of, the secreted protein having a continuous series of deleted residues from the amino or the carboxyl terminus, or both. Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-362 where m is an integer from 2 to 356, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:278. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: K-23 to L-362; S-24 to L-362; D-25 to L-362; A-26 to L-362; K-27 to L-362; K-28 to L-362; A-29 to L-362; A-30 to L-362; S-31 to L-362; K-32 to L-362; T-33 to L-362; L-34 to L-362; L-35 to L-362; E-36 to L-362; K-37 to L-362; S-38 to L-362; Q-39 to L-362; F-40 to L-362; S-41 to L-362; D-42 to L-362; K-43 to L-362; P-44 to L-362; V-45 to L-362; Q-46 to L-362; D-47 to L-362; R-48 to L-362; G-49 to L-362; L-50 to L-362; V-51 to L-362; V-52 to L-362; T-53 to L-362; D-54 to L-362; L-55 to L-362; K-56 to L-362; A-57 to L-362; E-58 to L-362; S-59 to L-362; V-60 to L-362; V-61 to L-362; L-62 to L-362; E-63 to L-362; H-64 to L-362; R-65 to L-362; S-66 to L-362; Y-67 to L-362; C-68 to L-362; S-69 to L-362; A-70 to L-362; K-71 to L-362; A-72 to L-362; R-73 to L-362; D-74 to L-362; R-75 to L-362; H-76 to L-362; F-77 to L-362; A-78 to L-362; G-79 to L-362; D-80 to L-362; V-81 to L-362; L-82 to L-362; G-83 to L-362; Y-84 to L-362; V-85 to L-362; T-86 to L-362; P-87 to L-362; W-88 to L-362; N-89 to L-362; S-90 to L-362; H-91 to L-362; G-92 to L-362; Y-93 to L-362; D-94 to L-362; V-95 to L-362; T-96 to L-362; K-97 to L-362; V-98 to L-362; F-99 to L-362; G-100 to L-362; S-101 to L-362; K-





262 to L-362; K-263 to L-362; I-264 to L-362; L-265 to L-362; L-266 to L-362; G-267 to L-362; L-268 to L-362; N-269 to L-362; F-270 to L-362; Y-271 to L-362; G-272 to L-362; M-273 to L-362; D-274 to L-362; Y-275 to L-362; A-276 to L-362; T-277 to L-362; S-278 to L-362; K-279 to L-362; D-280 to L-362; A-281 to L-362; R-282 to L-362; E-283 to L-362; P-284 to L-362; V-285 to L-362; V-286 to L-362; G-287 to L-362; A-288 to L-362; R-289 to L-362; Y-290 to L-362; I-291 to L-362; Q-292 to L-362; T-293 to L-362; L-294 to L-362; K-295 to L-362; D-296 to L-362; H-297 to L-362; R-298 to L-362; P-299 to L-362; R-300 to L-362; M-301 to L-362; V-302 to L-362; W-303 to L-362; D-304 to L-362; S-305 to L-362; Q-306 to L-362; X-307 to L-362; S-308 to L-362; E-309 to L-362; H-310 to L-362; F-311 to L-362; F-312 to L-362; E-313 to L-362; Y-314 to L-362; K-315 to L-362; K-316 to L-362; S-317 to L-362; R-318 to L-362; S-319 to L-362; G-320 to L-362; R-321 to L-362; H-322 to L-362; V-323 to L-362; V-324 to L-362; F-325 to L-362; Y-326 to L-362; P-327 to L-362; T-328 to L-362; L-329 to L-362; K-330 to L-362; S-331 to L-362; L-332 to L-362; Q-333 to L-362; V-334 to L-362; R-335 to L-362; L-336 to L-362; E-337 to L-362; L-338 to L-362; A-339 to L-362; R-340 to L-362; E-341 to L-362; L-342 to L-362; G-343 to L-362; V-344 to L-362; G-345 to L-362; V-346 to L-362; S-347 to L-362; I-348 to L-362; W-349 to L-362; E-350 to L-362; L-351 to L-362; G-352 to L-362; Q-353 to L-362; G-354 to L-362; L-355 to L-362; D-356 to L-362; and Y-357 to L-362 of SEQ ID NO:278. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: R-2 to H-307; T-3 to H-307; L-4 to H-307; F-5 to H-307; N-6 to H-307; L-7 to H-307; L-8 to H-307; W-9 to H-307; L-10 to H-307; A-11 to H-307; L-12 to H-307; A-13 to H-307; C-14 to H-307; S-15 to H-307; P-16 to H-307; V-17 to H-307; H-18 to H-307; T-19 to H-307; T-20 to H-307; L-21 to H-307; S-22 to H-307; K-23 to H-307; S-24 to H-307; D-25 to H-307; A-26 to H-307; K-27 to H-307; K-28 to H-307; A-29 to H-307; A-30 to H-307; S-31 to H-307; K-32 to H-307; T-33 to H-307; L-34 to H-307; L-35 to H-307; E-36 to H-307; K-37 to H-307; S-38 to H-307; Q-39 to H-307; F-40 to H-307; S-41 to H-307; D-42 to H-307; K-43 to H-307; P-44 to H-307; V-45 to H-307; Q-46 to H-307; D-47 to H-307; R-48 to H-307; G-49 to H-307;

L-50 to H-307; V-51 to H-307; V-52 to H-307; T-53 to H-307; D-54 to H-307; L-55 to H-307; K-56 to H-307; A-57 to H-307; E-58 to H-307; S-59 to H-307; V-60 to H-307; V-61 to H-307; L-62 to H-307; E-63 to H-307; H-64 to H-307; R-65 to H-307; S-66 to H-307; Y-67 to H-307; C-68 to H-307; S-69 to H-307; A-70 to H-307; K-71 to H-307; A-72 to H-307; R-73 to H-307; D-74 to H-307; R-75 to H-307; H-76 to H-307; F-77 to H-307; A-78 to H-307; G-79 to H-307; D-80 to H-307; V-81 to H-307; L-82 to H-307; G-83 to H-307; Y-84 to H-307; V-85 to H-307; T-86 to H-307; P-87 to H-307; W-88 to H-307; N-89 to H-307; S-90 to H-307; H-91 to H-307; G-92 to H-307; Y-93 to H-307; D-94 to H-307; V-95 to H-307; T-96 to H-307; K-97 to H-307; V-98 to H-307; F-99 to H-307; G-100 to H-307; S-101 to H-307; K-102 to H-307; F-103 to H-307; T-104 to H-307; Q-105 to H-307; I-106 to H-307; S-107 to H-307; P-108 to H-307; V-109 to H-307; W-110 to H-307; L-111 to H-307; Q-112 to H-307; L-113 to H-307; K-114 to H-307; R-115 to H-307; R-116 to H-307; G-117 to H-307; R-118 to H-307; E-119 to H-307; M-120 to H-307; F-121 to H-307; E-122 to H-307; V-123 to H-307; T-124 to H-307; G-125 to H-307; L-126 to H-307; H-127 to H-307; D-128 to H-307; V-129 to H-307; D-130 to H-307; Q-131 to H-307; G-132 to H-307; W-133 to H-307; M-134 to H-307; R-135 to H-307; A-136 to H-307; V-137 to H-307; R-138 to H-307; K-139 to H-307; H-140 to H-307; A-141 to H-307; K-142 to H-307; G-143 to H-307; L-144 to H-307; H-145 to H-307; I-146 to H-307; V-147 to H-307; P-148 to H-307; R-149 to H-307; L-150 to H-307; L-151 to H-307; F-152 to H-307; E-153 to H-307; D-154 to H-307; W-155 to H-307; T-156 to H-307; Y-157 to H-307; D-158 to H-307; D-159 to H-307; F-160 to H-307; R-161 to H-307; N-162 to H-307; V-163 to H-307; L-164 to H-307; D-165 to H-307; S-166 to H-307; E-167 to H-307; D-168 to H-307; E-169 to H-307; I-170 to H-307; E-171 to H-307; E-172 to H-307; L-173 to H-307; S-174 to H-307; K-175 to H-307; T-176 to H-307; V-177 to H-307; V-178 to H-307; Q-179 to H-307; V-180 to H-307; A-181 to H-307; K-182 to H-307; N-183 to H-307; Q-184 to H-307; H-185 to H-307; F-186 to H-307; D-187 to H-307; G-188 to H-307; F-189 to H-307; V-190 to H-307; V-191 to H-307; E-192 to H-307; V-193 to H-307; W-194 to H-307; N-195 to H-307; Q-196 to H-307; L-197 to H-307; L-198 to H-307; S-199 to H-307; Q-200 to H-307; K-201 to H-307; R-202 to H-307; V-203 to H-307; G-204 to H-307; L-205 to H-307; I-206 to H-307; H-207 to H-307; M-208 to H-307; L-209 to H-307; T-210 to H-307; H-211 to H-307; L-212 to H-307; T-213 to H-307; V-214 to H-307; W-215 to H-307; Y-216 to H-307; D-217 to H-307; D-218 to H-307; F-219 to H-307; R-220 to H-307; N-221 to H-307; V-222 to H-307; L-223 to H-307; D-224 to H-307; S-225 to H-307; E-226 to H-307; I-227 to H-307; E-228 to H-307; L-229 to H-307; S-230 to H-307; K-231 to H-307; T-232 to H-307; V-233 to H-307; Q-234 to H-307; V-235 to H-307; A-236 to H-307; K-237 to H-307; N-238 to H-307; Q-239 to H-307; H-240 to H-307; F-241 to H-307; D-242 to H-307; G-243 to H-307; F-244 to H-307; V-245 to H-307; V-246 to H-307; E-247 to H-307; V-248 to H-307; W-249 to H-307; N-250 to H-307; Q-251 to H-307; L-252 to H-307; L-253 to H-307; S-254 to H-307; Q-255 to H-307; K-256 to H-307; R-257 to H-307; V-258 to H-307; G-259 to H-307; L-260 to H-307; I-261 to H-307; H-262 to H-307; M-263 to H-307; L-264 to H-307; T-265 to H-307; H-266 to H-307; L-267 to H-307; T-268 to H-307; V-269 to H-307; W-270 to H-307; Y-271 to H-307; D-272 to H-307; D-273 to H-307; F-274 to H-307; R-275 to H-307; N-276 to H-307; V-277 to H-307; L-278 to H-307; D-279 to H-307; S-280 to H-307; E-281 to H-307; I-282 to H-307; E-283 to H-307; L-284 to H-307; S-285 to H-307; Q-286 to H-307; K-287 to H-307; R-288 to H-307; V-289 to H-307; G-290 to H-307; L-291 to H-307; I-292 to H-307; H-293 to H-307; M-294 to H-307; L-295 to H-307; T-296 to H-307; H-297 to H-307; L-298 to H-307; T-299 to H-307; V-300 to H-307; W-301 to H-307; Y-302 to H-307; D-303 to H-307; D-304 to H-307; F-305 to H-307; R-306 to H-307; N-307 to H-307; V-308 to H-307; L-309 to H-307; D-310 to H-307; S-311 to H-307; E-312 to H-307; I-313 to H-307; E-314 to H-307; L-315 to H-307; S-316 to H-307; Q-317 to H-307; K-318 to H-307; R-319 to H-307; V-320 to H-307; G-321 to H-307; L-322 to H-307; I-323 to H-307; H-324 to H-307; M-325 to H-307; L-326 to H-307; T-327 to H-307; H-328 to H-307; L-329 to H-307; T-330 to H-307; V-331 to H-307; W-332 to H-307; Y-333 to H-307; D-334 to H-307; D-335 to H-307; F-336 to H-307; R-337 to H-307; N-338 to H-307; V-339 to H-307; L-340 to H-307; D-341 to H-307; S-342 to H-307; E-343 to H-307; I-344 to H-307; E-345 to H-307; L-346 to H-307; S-347 to H-307; Q-348 to H-307; K-349 to H-307; R-350 to H-307; V-351 to H-307; G-352 to H-307; L-353 to H-307; I-354 to H-307; H-355 to H-307; M-356 to H-307; L-357 to H-307; T-358 to H-307; H-359 to H-307; L-360 to H-307; T-361 to H-307; V-362 to H-307; W-363 to H-307; Y-364 to H-307; D-365 to H-307; D-366 to H-307; F-367 to H-307; R-368 to H-307; N-369 to H-307; V-370 to H-307; L-371 to H-307; D-372 to H-307; S-373 to H-307; E-374 to H-307; I-375 to H-307; E-376 to H-307; L-377 to H-307; S-378 to H-307; Q-379 to H-307; K-380 to H-307; R-381 to H-307; V-382 to H-307; G-383 to H-307; L-384 to H-307; I-385 to H-307; H-386 to H-307; M-387 to H-307; L-388 to H-307; T-389 to H-307; H-390 to H-307; L-391 to H-307; T-392 to H-307; V-393 to H-307; W-394 to H-307; Y-395

H-307; A-213 to H-307; E-214 to H-307; A-215 to H-307; L-216 to H-307; H-217 to H-307; Q-218 to H-307; A-219 to H-307; R-220 to H-307; L-221 to H-307; L-222 to H-307; A-223 to H-307; L-224 to H-307; L-225 to H-307; V-226 to H-307; I-227 to H-307; P-228 to H-307; P-229 to H-307; A-230 to H-307; I-231 to H-307; T-232 to H-307; P-233 to H-307; G-234 to H-307; T-235 to H-307; D-236 to H-307; Q-237 to H-307; L-238 to H-307; G-239 to H-307; M-240 to H-307; F-241 to H-307; T-242 to H-307; H-243 to H-307; K-244 to H-307; E-245 to H-307; F-246 to H-307; E-247 to H-307; Q-248 to H-307; L-249 to H-307; A-250 to H-307; P-251 to H-307; V-252 to H-307; L-253 to H-307; D-254 to H-307; G-255 to H-307; F-256 to H-307; S-257 to H-307; L-258 to H-307; M-259 to H-307; T-260 to H-307; Y-261 to H-307; D-262 to H-307; Y-263 to H-307; S-264 to H-307; T-265 to H-307; A-266 to H-307; H-267 to H-307; Q-268 to H-307; P-269 to H-307; G-270 to H-307; P-271 to H-307; N-272 to H-307; A-273 to H-307; P-274 to H-307; L-275 to H-307; S-276 to H-307; W-277 to H-307; V-278 to H-307; R-279 to H-307; A-280 to H-307; C-281 to H-307; V-282 to H-307; Q-283 to H-307; V-284 to H-307; L-285 to H-307; D-286 to H-307; P-287 to H-307; K-288 to H-307; S-289 to H-307; K-290 to H-307; W-291 to H-307; R-292 to H-307; S-293 to H-307; K-294 to H-307; I-295 to H-307; L-296 to H-307; L-297 to H-307; G-298 to H-307; L-299 to H-307; N-300 to H-307; F-301 to H-307; and Y-302 to H-307 of SEQ ID NO: 1232. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to inhibit the Mixed Lymphocyte Reaction), other functional activities (e.g., biological activities, ability to multimerize, ability to bind ligand, ability to generate antibodies, ability to bind antibodies) may still be retained. For example the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a

large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxyl terminus of the amino acid sequence of the polypeptide shown in Figures 1A-B (SEQ ID NO:278), as described by the general formula 1-n, where n is an integer from 6 to 356, where n corresponds to the position of the amino acid residue identified in SEQ ID NO:278. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: K-23 to L-362; K-23 to L-361; K-23 to D-360; K-23 to Y-359; K-23 to F-358; K-23 to Y-357; K-23 to D-356; K-23 to L-355; K-23 to G-354; K-23 to Q-353; K-23 to G-352; K-23 to L-351; K-23 to E-350; K-23 to W-349; K-23 to I-348; K-23 to S-347; K-23 to V-346; K-23 to G-345; K-23 to V-344; K-23 to G-343; K-23 to L-342; K-23 to E-341; K-23 to R-340; K-23 to A-339; K-23 to L-338; K-23 to E-337; K-23 to L-336; K-23 to R-335; K-23 to V-334; K-23 to Q-333; K-23 to L-332; K-23 to S-331; K-23 to K-330; K-23 to L-329; K-23 to T-328; K-23 to P-327; K-23 to Y-326; K-23 to F-325; K-23 to V-324; K-23 to V-323; K-23 to H-322; K-23 to R-321; K-23 to G-320; K-23 to S-319; K-23 to R-318; K-23 to S-317; K-23 to K-316; K-23 to K-315; K-23 to Y-314; K-23 to E-313; K-23 to F-312; K-23 to F-311; K-23 to H-310; K-23 to E-309; K-23 to S-308; K-23 to X-307; K-23 to Q-306; K-23 to S-305; K-23 to D-304; K-23 to W-303; K-23 to V-302; K-23 to M-301; K-23 to R-300; K-23 to P-299; K-23 to R-298; K-23 to H-297; K-23 to D-296; K-23 to K-295; K-23 to L-294; K-23 to T-293; K-23 to Q-292; K-23 to I-291; K-23 to Y-290; K-23 to R-289; K-23 to A-288; K-23 to G-287; K-23 to V-286; K-23 to V-285; K-23 to P-284; K-23 to E-283; K-23 to R-282; K-23 to A-281; K-23 to D-280; K-23 to K-279; K-23 to S-278; K-23 to T-277; K-23 to A-276; K-23 to Y-275; K-23 to D-274; K-23 to M-273; K-23 to G-272; K-23 to Y-271; K-23 to F-270; K-23 to N-269; K-23 to L-268; K-23 to G-267; K-23 to L-266; K-23 to L-265; K-23 to I-264; K-23 to K-263; K-23 to S-262; K-23 to R-261; K-23 to W-260; K-23 to K-259; K-23 to S-258; K-23 to K-257; K-23 to P-256; K-23 to D-255; K-23 to L-254; K-23 to V-253; K-23 to Q-252; K-23 to V-251; K-23 to C-250; K-23 to A-249; K-23 to R-248; K-23 to V-247; K-23 to W-246; K-23 to S-245;

K-23 to L-244; K-23 to P-243; K-23 to A-242; K-23 to N-241; K-23 to P-240; K-23  
 to G-239; K-23 to P-238; K-23 to Q-237; K-23 to H-236; K-23 to A-235; K-23 to T-  
 234; K-23 to S-233; K-23 to Y-232; K-23 to D-231; K-23 to Y-230; K-23 to T-229;  
 K-23 to M-228; K-23 to L-227; K-23 to S-226; K-23 to F-225; K-23 to G-224; K-23  
 5 to D-223; K-23 to L-222; K-23 to V-221; K-23 to P-220; K-23 to A-219; K-23 to L-  
 218; K-23 to Q-217; K-23 to E-216; K-23 to F-215; K-23 to E-214; K-23 to K-213;  
 K-23 to H-212; K-23 to T-211; K-23 to F-210; K-23 to M-209; K-23 to G-208; K-23  
 to L-207; K-23 to Q-206; K-23 to D-205; K-23 to T-204; K-23 to V-203; K-23 to R-  
 202; K-23 to K-201; K-23 to Q-200; K-23 to S-199; K-23 to L-198; K-23 to L-197;  
 10 K-23 to Q-196; K-23 to N-195; K-23 to W-194; K-23 to V-193; K-23 to E-192; K-23  
 to V-191; K-23 to V-190; K-23 to F-189; K-23 to G-188; K-23 to D-187; K-23 to F-  
 186; K-23 to H-185; K-23 to Q-184; K-23 to N-183; K-23 to K-182; K-23 to A-181;  
 K-23 to V-180; K-23 to Q-179; K-23 to V-178; K-23 to V-177; K-23 to T-176; K-23  
 to K-175; K-23 to S-174; K-23 to L-173; K-23 to E-172; K-23 to E-171; K-23 to I-  
 15 170; K-23 to E-169; K-23 to D-168; K-23 to E-167; K-23 to S-166; K-23 to D-165;  
 K-23 to L-164; K-23 to V-163; K-23 to N-162; K-23 to R-161; K-23 to F-160; K-23  
 to D-159; K-23 to D-158; K-23 to Y-157; K-23 to T-156; K-23 to W-155; K-23 to D-  
 154; K-23 to E-153; K-23 to F-152; K-23 to L-151; K-23 to L-150; K-23 to R-149;  
 K-23 to P-148; K-23 to V-147; K-23 to I-146; K-23 to H-145; K-23 to L-144; K-23 to  
 20 G-143; K-23 to K-142; K-23 to A-141; K-23 to H-140; K-23 to K-139; K-23 to R-  
 138; K-23 to V-137; K-23 to A-136; K-23 to R-135; K-23 to M-134; K-23 to W-133;  
 K-23 to G-132; K-23 to Q-131; K-23 to D-130; K-23 to V-129; K-23 to D-128; K-23  
 to H-127; K-23 to L-126; K-23 to G-125; K-23 to T-124; K-23 to V-123; K-23 to E-  
 122; K-23 to F-121; K-23 to M-120; K-23 to E-119; K-23 to R-118; K-23 to G-117;  
 25 K-23 to R-116; K-23 to R-115; K-23 to K-114; K-23 to L-113; K-23 to Q-112; K-23  
 to L-111; K-23 to W-110; K-23 to V-109; K-23 to P-108; K-23 to S-107; K-23 to I-  
 106; K-23 to Q-105; K-23 to T-104; K-23 to F-103; K-23 to K-102; K-23 to S-101;  
 K-23 to G-100; K-23 to F-99; K-23 to V-98; K-23 to K-97; K-23 to T-96; K-23 to V-  
 95; K-23 to D-94; K-23 to Y-93; K-23 to G-92; K-23 to H-91; K-23 to S-90; K-23 to  
 30 N-89; K-23 to W-88; K-23 to P-87; K-23 to T-86; K-23 to V-85; K-23 to Y-84; K-23  
 to G-83; K-23 to L-82; K-23 to V-81; K-23 to D-80; K-23 to G-79; K-23 to A-78; K-  
 23 to F-77; K-23 to H-76; K-23 to R-75; K-23 to D-74; K-23 to R-73; K-23 to A-72;

K-23 to K-71; K-23 to A-70; K-23 to S-69; K-23 to C-68; K-23 to Y-67; K-23 to S-66; K-23 to R-65; K-23 to H-64; K-23 to E-63; K-23 to L-62; K-23 to V-61; K-23 to V-60; K-23 to S-59; K-23 to E-58; K-23 to A-57; K-23 to K-56; K-23 to L-55; K-23 to D-54; K-23 to T-53; K-23 to V-52; K-23 to V-51; K-23 to L-50; K-23 to G-49; K-23 to R-48; K-23 to D-47; K-23 to Q-46; K-23 to V-45; K-23 to P-44; K-23 to K-43; K-23 to D-42; K-23 to S-41; K-23 to F-40; K-23 to Q-39; K-23 to S-38; K-23 to K-37; K-23 to E-36; K-23 to L-35; K-23 to L-34; K-23 to T-33; K-23 to K-32; K-23 to S-31; K-23 to A-30; and K-23 to A-29 of SEQ ID NO:278. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: K-23 to R-306; K-23 to S-305; K-23 to T-304; K-23 to G-303; K-23 to Y-302; K-23 to F-301; K-23 to N-300; K-23 to L-299; K-23 to G-298; K-23 to L-297; K-23 to L-296; K-23 to I-295; K-23 to K-294; K-23 to S-293; K-23 to R-292; K-23 to W-291; K-23 to K-290; K-23 to S-289; K-23 to K-288; K-23 to P-287; K-23 to D-286; K-23 to L-285; K-23 to V-284; K-23 to Q-283; K-23 to V-282; K-23 to C-281; K-23 to A-280; K-23 to R-279; K-23 to V-278; K-23 to W-277; K-23 to S-276; K-23 to L-275; K-23 to P-274; K-23 to A-273; K-23 to N-272; K-23 to P-271; K-23 to G-270; K-23 to P-269; K-23 to Q-268; K-23 to H-267; K-23 to A-266; K-23 to T-265; K-23 to S-264; K-23 to Y-263; K-23 to D-262; K-23 to Y-261; K-23 to T-260; K-23 to M-259; K-23 to L-258; K-23 to S-257; K-23 to F-256; K-23 to G-255; K-23 to D-254; K-23 to L-253; K-23 to V-252; K-23 to P-251; K-23 to A-250; K-23 to L-249; K-23 to Q-248; K-23 to E-247; K-23 to F-246; K-23 to E-245; K-23 to K-244; K-23 to H-243; K-23 to T-242; K-23 to F-241; K-23 to M-240; K-23 to G-239; K-23 to L-238; K-23 to Q-237; K-23 to D-236; K-23 to T-235; K-23 to G-234; K-23 to P-233; K-23 to T-232; K-23 to I-231; K-23 to A-230; K-23 to P-229; K-23 to P-228; K-23 to I-227; K-23 to V-226; K-23 to L-225; K-23 to L-224; K-23 to A-223; K-23 to L-222; K-23 to L-221; K-23 to R-220; K-23 to A-219; K-23 to Q-218; K-23 to H-217; K-23 to L-216; K-23 to A-215; K-23 to E-214; K-23 to A-213; K-23 to L-212; K-23 to H-211; K-23 to T-210; K-23 to L-209; K-23 to M-208; K-23 to H-207; K-23 to I-206; K-23 to L-205; K-23 to G-204; K-23 to V-203; K-23 to R-202; K-23 to K-201; K-23 to Q-200; K-23 to S-199; K-23 to L-198; K-23 to L-197; K-23 to Q-196; K-23 to N-

195; K-23 to W-194; K-23 to V-193; K-23 to E-192; K-23 to V-191; K-23 to V-190; K-23 to F-189; K-23 to G-188; K-23 to D-187; K-23 to F-186; K-23 to H-185; K-23 to Q-184; K-23 to N-183; K-23 to K-182; K-23 to A-181; K-23 to V-180; K-23 to Q-179; K-23 to V-178; K-23 to V-177; K-23 to T-176; K-23 to K-175; K-23 to S-174; K-23 to L-173; K-23 to E-172; K-23 to E-171; K-23 to I-170; K-23 to E-169; K-23 to D-168; K-23 to E-167; K-23 to S-166; K-23 to D-165; K-23 to L-164; K-23 to V-163; K-23 to N-162; K-23 to R-161; K-23 to F-160; K-23 to D-159; K-23 to D-158; K-23 to Y-157; K-23 to T-156; K-23 to W-155; K-23 to D-154; K-23 to E-153; K-23 to F-152; K-23 to L-151; K-23 to L-150; K-23 to R-149; K-23 to P-148; K-23 to V-147; K-23 to I-146; K-23 to H-145; K-23 to L-144; K-23 to G-143; K-23 to K-142; K-23 to A-141; K-23 to H-140; K-23 to K-139; K-23 to R-138; K-23 to V-137; K-23 to A-136; K-23 to R-135; K-23 to M-134; K-23 to W-133; K-23 to G-132; K-23 to Q-131; K-23 to D-130; K-23 to V-129; K-23 to D-128; K-23 to H-127; K-23 to L-126; K-23 to G-125; K-23 to T-124; K-23 to V-123; K-23 to E-122; K-23 to F-121; K-23 to M-120; K-23 to E-119; K-23 to R-118; K-23 to G-117; K-23 to R-116; K-23 to R-115; K-23 to K-114; K-23 to L-113; K-23 to Q-112; K-23 to L-111; K-23 to W-110; K-23 to V-109; K-23 to P-108; K-23 to S-107; K-23 to I-106; K-23 to Q-105; K-23 to T-104; K-23 to F-103; K-23 to K-102; K-23 to S-101; K-23 to G-100; K-23 to F-99; K-23 to V-98; K-23 to K-97; K-23 to T-96; K-23 to V-95; K-23 to D-94; K-23 to Y-93; K-23 to G-92; K-23 to H-91; K-23 to S-90; K-23 to N-89; K-23 to W-88; K-23 to P-87; K-23 to T-86; K-23 to V-85; K-23 to Y-84; K-23 to G-83; K-23 to L-82; K-23 to V-81; K-23 to D-80; K-23 to G-79; K-23 to A-78; K-23 to F-77; K-23 to H-76; K-23 to R-75; K-23 to D-74; K-23 to R-73; K-23 to A-72; K-23 to K-71; K-23 to A-70; K-23 to S-69; K-23 to C-68; K-23 to Y-67; K-23 to S-66; K-23 to R-65; K-23 to H-64; K-23 to E-63; K-23 to L-62; K-23 to V-61; K-23 to V-60; K-23 to S-59; K-23 to E-58; K-23 to A-57; K-23 to K-56; K-23 to L-55; K-23 to D-54; K-23 to T-53; K-23 to V-52; K-23 to V-51; K-23 to L-50; K-23 to G-49; K-23 to R-48; K-23 to D-47; K-23 to Q-46; K-23 to V-45; K-23 to P-44; K-23 to K-43; K-23 to D-42; K-23 to S-41; K-23 to F-40; K-23 to Q-39; K-23 to S-38; K-23 to K-37; K-23 to E-36; K-23 to L-35; K-23 to L-34; K-23 to T-33; K-23 to K-32; K-23 to S-31; K-23 to A-30; and K-23 to A-29 of SEQ ID NO:1232. Polypeptides encoded by these polynucleotides are also encompassed by the invention.



In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:278, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 209080, where this portion excludes any integer of amino acid residues from 1 to about 356 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 209080, or any integer of amino acid residues from 1 to about 356 amino acids from the carboxyl terminus, or any combination of the above amino terminal and carboxyl terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209080. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis. The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention have uses, such as, for example, as a marker in linkage analysis for chromosome 11.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include, but are not limited to, immune and gastrointestinal disorders and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

When tested against Jurkat cell lines, supernatants removed from cells expressing this gene activated the nuclear-factor kB (NF-kB) transcription factor. Thus, it is likely that this gene activates Jurkat cells by activating a transcriptional factor found within these cells. Nuclear factor kB is a transcription factor activated by a wide variety of agents, leading to cell activation, differentiation, or apoptosis. Reporter constructs utilizing the NF-kB promoter element were used to screen supernatants for such activity.

Additionally, products of this gene have been found to inhibit the Mixed Lymphocyte Reaction (MLR). This assay is described in Example 58 herein. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

The tissue distribution in immune cells (e.g., T-cells, macrophages) and inhibition of the MLR indicates that the polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, treatment, and/or prevention of many diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus

erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma. Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore, polynucleotides and polypeptides of the invention (including fragments, variants, and derivatives) may be also used to treat, prevent and/or diagnose immunological disorders including, but not limited to, arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma.

The tissue distribution in human T-cells and human colon carcinoma indicates that the polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, treatment, and/or prevention of immune disorders and gastrointestinal diseases. Non-limiting representative uses for these polynucleotides and polypeptides are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may as be useful as a tumor marker and/or immunotherapy targets for the above listed tissues. In addition, polynucleotides and polypeptides of the invention may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, in the differentiation and/or proliferation of various cell types (e.g., T, B and natural killer lymphocytes, monocytes, dendritic cells), modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, and/or modulation of cytokine production by accessory cells.

Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1501 of SEQ ID NO:40, b is an integer of 15 to 1515, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 31**

The translation product of this gene shares sequence homology with Ribosomal protein L11 of *Caenorhabditis elegans*. (See Genbank Accession No. 156201.)

20 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
ERGV SINQFCKEFNERTKDIKEGIPLPTKILVKPDRTFEIKIGQPTVSYFLKAAA  
GI EKGARQTGKEVAGLVTLKHVYEIARIKAQDEAFALQDVPLSSVVR SIIG  
SARSLGIRVVKDLSS EELAAF QKERAIFLAAQKEADLAAQE EAAKK (SEQ ID  
25 NO:564), ERGV SINQFCKEFNERTKDIKEGIPLPTKILVKPDRTFEIKIGQ  
PTVSYFL (SEQ ID NO:565), KAAAGIEKGARQTGKEVAGLVTLKHVYEIARIK  
AQDEAFALQDVPLSSV (SEQ ID NO:566), and/or VRSIIGSARSLGIRVVK  
DLSSEELAAFQKERAIFLAAQKEADLAAQE EAAKK (SEQ ID NO:567).

Moreover, fragments and variants of these polypeptides (such as, for example,  
30 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed in human embryo tissue, and to a lesser extent, in human epithelioid sarcoma.

- Therefore, polynucleotides and polypeptides of the invention are useful as reagents for identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, development disorders and epithelial cell cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the embryonic and epithelial cell systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. embryonic, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 279 as residues: Lys-34 to Gly-40.

- The tissue distribution in human embryo indicates that the protein product of this gene is useful for the diagnosis and treatment of developmental disorders and epithelial cancer. Furthermore, expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the

protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 690 of SEQ ID NO:41, b is an integer of 15 to 704, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 32**

This gene is expressed primarily in resting T-cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammatory and general immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in T-cells indicates that the protein product of this gene is useful for the diagnosis and treatment of disorders of the immune system.

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Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1080 of SEQ ID NO:42, b is an integer of 15 to 1094, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 33**

This gene is believed to reside on chromosome 1. Accordingly, polynucleotides derived from this gene are useful in linkage analysis as chromosome 1 markers.

This gene is expressed primarily in prostate, and to a lesser extent in soares adult brain, human umbilical vein endothelial cells, and amniotic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate-related disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential

identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the urinary system and nervous system expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. prostate, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in prostate indicates that the protein products of this gene are useful for the diagnosis and treatment of disorders of the urinary and nervous systems. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1807 of SEQ ID NO:43, b is an integer of 15 to 1821, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 34



This gene shares sequence homology with R05G6.4 gene product. (See Genbank Accession No. gi|1326338.) This gene also shares sequence homology with the cyclophilin-like protein CyP-60. (See Genbank Accession No. 1199598, see also Biochem. J. 314 (1), 313-319 (1996).)

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

AVYTYHEKKKDTAASGYGTQNIRLSRDAVKDFDCCCLSLQPCHDPVVTPDG  
 YLYEREAILLEYILHQKKEIARQMKAIEKQGRGTRREEQKELQRAASQDHVRGF  
 LEKESAIVSRPLNPFTAKALSGTSPDDVQPGPSVGPPSKDKDKVLPSFWIPSLT  
 10 PEAKATKLEKPSRTVTCMSGKPLRMSDLTPVHFTPLDSSVDRVGLITRSEY  
 VCAVTRDSLSNATPCAVLRPSGAVVTLECVEKLIRKDMVDPVTGDKLTDRDI  
 VLQGGT (SEQ ID NO:568),  
 YLYEREAILLEYILHQKKEIARQMKAIEKQGRGTRREEQKELQRAASQDHVRGF  
 LE (SEQ ID NO:569),  
 15 FTAKALSGTSPDDVQPGPSVGPPSKDKDKVLPSFWIPSLTPEAKATKLEKPSR  
 TVTCMSGKPL (SEQ ID NO:570),  
 VHFTPLDSSVDRVGLITRSEYVCAVTRDSLSNATPCAVLRPSGAVVTLECVE  
 KLI (SEQ ID NO:571), and/or  
 MSDLTTPVHFTPLDSSVDRVGLITRSEYVCAVTRDSLSNATPCAVLRPSGAVV  
 20 TLECVEKLIRKDM (SEQ ID NO:572).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 25 encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human testis, and to a lesser extent in activated T-cells.

30 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,

male reproductive disorders and in particular testicular cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the

5 reproductive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. testes, immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression  
10 level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human testis indicates that the protein product of this gene is useful for the diagnosis and treatment of disorders of the male reproductive system, and in particular of testicular cancer. Furthermore, this gene is useful for the

15 treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is  
20 believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone  
25 formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
30 related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

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cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1010 of SEQ ID NO:44, b is an integer of 15 to 1024, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 35

The translation product of this gene shares sequence homology with Lpe5p of *Saccharomyces cerevisiae*, which is thought to be important in the metabolism of phospholipids. The gene encoding the disclosed cDNA is thought to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

This gene is expressed primarily in liver and brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, metabolic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the metabolic and nervous systems expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. liver, brain, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 283 as residues: Pro-14 to Leu-20, Lys-28 to Asn-38, Arg-109 to Arg-114, Lys-119 to Asn-124, Glu-152 to Leu-157, or Pro-172 to Val-180.

The tissue distribution in liver and brain, combined with the homology to Lpe5p of *Saccharomyces cerevisiae* indicates that the protein product of this gene is useful for the diagnosis and treatment of metabolic and nervous disorders.

Additionally, the tissue distribution indicates that the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 969 of SEQ ID NO:45, b is an integer of 15 to 983, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.

#### *FEATURES OF PROTEIN ENCODED BY GENE NO: 36*

This gene shares sequence homology with the nuclear ribonucleoprotein U (HNRNP U), encoded by *C. elegans* (See Genbank Accession gi|1703576.).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MDTSENRPENDVPEPPMPLADQVSNDDRPEGSVEDEEKKESSLPKSFKRKISV  
VSATKGVPAGNSDTEGGQPGRKRRWGASTATTQKKPSISITTESLKSILPIDIKP  
LAGQEAVVDLHADDSETERNGDDGTHDKGLKICRTVTQVVPAEGQE  
NGQREEEEEKEPEAEPPVPPQVSVEVALPPPAEHEVKKVTLGDTLTRRSISQ  
QKSGVSITIDDPVRTAQVSPPRGKISNIVHISNLVRPFTLGQLKELLGRTGTLV

EEAFWIDKIKSHCFVTYSTVEEAVATRTALHGVKWPQSNPKFLCADYAEQDE  
 LDYHRGLLVDRPSETKTEEQGIPRPLHPPPPPVQPPQHPRAEQREQERAVRE  
 QWAEREREMERRERTRSEREWDRDKVREGPRSRSRXRRRKERAKSKEK  
 KSEKKEKAQEPPAKLLDDLFRKTKAAPCIYWLPLTDSQIVQKEAERAERAK  
 5 EREKRRKEQEEEEQKEREKEAERERNRQLEREKRREHSRERDRERERERERD  
 RGDRDRDRERDRERGRERDRRDTKRHSRSRSRSTPVRDRGGR (SEQ ID  
 NO:573),

ENDVPEPPMPIADQVSNDDRPEGSVEDEEKKESLPSFKRKISVVSA (SEQ ID  
 NO:574), VDLHADDRISEDETERNGDDGTHDKGLKICRTVTQV (SEQ ID

10 NO:575),

PQVSVEVALPPAEHEVKKVTLGDTLTRRSISQQKSGVSITIDDPVRTAQVPSP  
 P (SEQ ID NO:576),

LKELLGRTGTLVEEAFWIDKIKSHCFVTYSTVEEAVATRTALHGVKWPQSNP  
 KFL (SEQ ID NO:577),

15 VDRPSETKTEEQGIPRPLHPPPPPVQPPQHPRAEQREQERAVREQWAERERE  
 (SEQ ID NO:578),

EWDRDKVREGPRSRSRXRRRKERAKSKEKKSEKKEKAQEPPAKLLDDLFR  
 RKTKAAP (SEQ ID NO:579), LDVPLASRSPEFPLPLMTQSELPRCPPHPGAR  
 (SEQ ID NO:581), LATLSISPIWSVLSL (SEQ ID NO:582), and

20 PLTDSQIVQKEAERAERAKEREKRRKEQEEEEQKEREKEAERERNRQLEREK  
 RREHSRERDRER (SEQ ID NO:580). Moreover, fragments and variants of these

polypeptides (such as, for example, fragments as described herein, polypeptides at  
 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
 and polypeptides encoded by the polynucleotide which hybridizes, under stringent

25 conditions, to the polynucleotide encoding these polypeptides ) are encompassed by  
 the invention. Antibodies that bind polypeptides of the invention are also  
 encompassed by the invention. Polynucleotides encoding these polypeptides are also  
 encompassed by the invention.

An additional embodiment is the polynucleotides encoding these polypeptides.

30 The gene encoding the disclosed cDNA is thought to reside on chromosome 14.  
 Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 14.

This gene is expressed primarily in epididymus, and to a lesser extent in testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases of the male reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. epididymus, testes, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in epididymus and testes indicates that the protein product of this gene is useful for the diagnosis and treatment of male reproductive disorders. Furthermore, the protein product of this gene is useful for the treatment and diagnosis of conditions concerning proper reproductive and testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 37**

This gene is expressed primarily in amygdala.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammatory diseases and reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the amygdala, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in amygdala indicates that the protein product of this gene is useful for the diagnosis and treatment of inflammatory diseases and neural disorders. The amygdala processes sensory information and relays this to other areas of the brain including the endocrine and autonomic domains of the hypothalamus and the brain stem. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 826 of SEQ ID NO:47, b is an integer of 15 to 840, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 38

This gene shares sequence homology with human opsonin protein P35 fragment. (See Genbank Accession No. R94181.) The opsonin protein activates the phagocytosis of pathogenic microbes by phagocytic cells which indicates that the protein product of this gene may be useful in the treatment and/or prevention of a variety of immune conditions, particularly bacterial infections and antigen presentation.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: GCDSCPHLPREAFAQDTQAEGECSSRAERADMCPDAPPSQEVPEGPGAAP (SEQ ID NO:583), RGWLPSSCLSCALRVCPDSSSTQAMGMLLAFWLPGASWQEAARGQYSEDED TDTDEYKEAKASINPVTGRVEEKPPNPMEGMTEEQKEHEA (SEQ ID NO:584), and/or TQAMGMLLAFWLPGASWQEAARGQYSE (SEQ ID NO:585). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in immune-related tissues such as thymus, macrophage, and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders and infectious diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene

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at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 286 as residues: Lys-9 to Arg-14, or Met-38 to Asp-51.

The tissue distribution in immune tissues, particularly macrophages, combined with the homology to a conserved human opsonin protein indicates that the protein product of this gene is useful for diagnosis and treatment of immune disorders, as well as the treatment and/or diagnosis of infectious disease. Moreover, the gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
 5 formula of a-b, where a is any integer between 1 to 2418 of SEQ ID NO:48, b is an integer of 15 to 2432, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

# 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 39

The translation product of this gene shares sequence homology with alpha-2 type I collagen which is thought to be important in tissue repair. (See, e.g., Genbank Accession No. 211607.)

15 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 PQLPSCGRPWPGTASVFQSHTQGPREDPDP CRAQGSAGTHCPISLSPPRQ (SEQ ID NO:586),  
 KTHPRALWSAGPSCALCPGGSGXTSPPQGAPRGIXWDRCPQIQVLEGQRVRF  
 20 PSQPQHPSHLAPRGGCGWRPDSRPLLPTPSGLSSFFPLDA QCWPWRTVSWR (SEQ ID NO:587),  
 AGAPGQQARLQYLLSFQGE GAPHEXGATGEGGDGAWEACXCXRCLLNWQA  
 GGWGLQLSLMWLHRGPLRPPGVRWTPWAFLEACSWGPA LSLGSGHSLPGT  
 HEQAAWSRGCQH GQSPTQKCKSSKEPLAQAPPWDSPA APPHQGFADVLER  
 25 PTLEPFGVLAPPVPSALVEAAXQVLLREPQGGFXGTAAHRSRCWK GSG (SEQ ID NO:588),  
 MQLLFLPHPSQLHASLPHSAALPCPRGESLTTASPAGAAGR XDAVPRCRH  
 QAGRGWVPRGPCERGGGDRGKPRAVAWDXGSLRWAVWSARAGQGRSSEP  
 APLASRRGYSTCCLSRGKGLPMRXGRRGRGVMVPGKPACAXGAC (SEQ ID  
 30 NO:589), QHPSHLAPRGGCGWRPDSRPLLPTPSGLSSFFPL (SEQ ID NO:590),  
 GVRWTPWAFLEACSWGPA LSLGSGHSLPG (SEQ ID NO:591),  
 WDSAPAAPP HQGFADVLERPTLEPFGVLA (SEQ ID NO:592), and/or

RSSEPAPLASRRGYSTCCLSRGKGL PMR (SEQ ID NO:593). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the brain, and to a lesser extent, in the kidney and thymus

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, brain, kidney, endocrine, hematopoietic, and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, kidney, and immune disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, urogenital, renal, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain and thymus, combined with the homology to an alpha-2 type I collagen protein indicates that the protein product of this gene is useful for the diagnosis and treatment of tissue repair, and brain, kidney, immune disorders. Moreover, this protein may also be important in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal

chondrodysplasia type Schmid. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1728 of SEQ ID NO:49, b is an integer of 15 to 1742, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 40

The translation product of this gene shares sequence homology with mini-collagen which is thought to be important in tissue repair and tumor metastasis, and potentially in cellular migration, attachment, and/or chemotaxis. (See Genbank Accession No. gnl|PID|d1006976.)

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

PGFRGPSGLGCSFFPRSLGRVLPPGCQRPGAHADSSPPPTP (SEQ ID NO:594).

Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 16. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 16.

This gene is expressed in ovarian cancer, and to a lesser extent, in dendritic cells and smooth muscle.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tumor metastasis, tissue repair, integumentary, reproductive, and/or immune disorders, particularly cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the tumor metastasis and tissue repair, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., integumentary, immune, hematopoietic, reproductive, ovarian, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 288 as residues: Asn-2 to His-11.

The tissue distribution in dendritic cells, combined with the homology to the mini-collagen gene indicates that the protein product of this gene is useful for diagnosis and treatment of tumor metastasis and tissue repair. Alternatively, this protein may also be important in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1473 of SEQ ID NO:50, b is an integer of 15 to 1487, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 41

This gene shares sequence homology with the HIV TAT protein. (See Genbank Accession No. 328416.)

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

EDLKKPDPASLRAASCGEGKKRKACKNCTCGLAEELEKEKSREQMSSQPKSA  
 CGNCYLGDFAFRASCOPYLGMPAFKPGEKVLLS (SEQ ID NO:595);  
 EDLKKPDPASLRAASCGEGKKRKACKNCTCGLAEELEKEKSREQMSSQPKSA  
 CGNCYLGDFAFRASCOPYLGMPAFKPGEKVLLSDSNLHD (SEQ ID NO:596);  
 CGNCYLGDFAFRASCOPYLGMPAFKPGEKVLLSDS (SEQ ID NO:597);  
 SCGEGKKRKACKNCTCGLAEELEKE (SEQ ID NO:598),  
 SQPKSACGNCYLGDFAFRASC (SEQ ID NO:599); CCCVSKDQGIMGPGFR  
 (SEQ ID NO:601),  
 HSVTELQTPALSLISAMLPPSCLSELLVYSILCDTSQVAHNLLRAPEDSLTGCC  
 DDIQCPSAPFHPQPHLTVALHLCPPVVIYVNLQVLNLLHILTYLEILHVL (SEQ  
 ID NO:602), LLVYSILCDTSQVAHNLLRAPEDS (SEQ ID NO:603),  
 LTVALHLCPPVVIYVNLQVLNLLHILT (SEQ ID NO:604), and/or  
 REAGQNSERQYVSLSRDP (SEQ ID NO:600). Moreover, fragments and variants  
 of these polypeptides (such as, for example, fragments as described herein,

polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the infant brain, and to a lesser extent, in the breast and testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neural, developmental, reproductive, brain, testes and breast disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the brain, testes and breast disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, reproductive, testicular, breast, and cancerous and wounded tissues) or bodily fluids (e.g., seminal fluid, amniotic fluid, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 289 as residues: Pro-7 to Val-15.

The tissue distribution in infant brain tissue indicates that the protein product of this gene is useful for diagnosis and treatment of neural and other related disorders. Similarly the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia,

obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular or reproductive system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1314 of SEQ ID NO:51, b is an integer of 15 to 1328, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 42

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

FFNALYVFRKPQAIKFDSEKENKRKNPTKYNNPLRYIYFKVKLIFQFIPLANYKI  
K (SEQ ID NO:605). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the



polynucleotide encoding this polypeptide are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

- 5           The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in the infant brain, human cerebellum, and to a lesser extent, in medulloblastoma.

- 10           Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, brain related disorders, such as neurodegenerative conditions, medulloblastoma, and other cancers or proliferative conditions. Similarly, polypeptides and antibodies  
15           directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain related disorders and brain cancers, including medulloblastoma, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural,  
20           developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- 25           Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 290 as residues: Thr-41 to Glu-47.

- The tissue distribution in infant brain and medulloblastoma indicates that the protein product of this gene is useful for diagnosis and treatment of human brain related disorders, brain cancers, and medulloblastoma. Similarly, the protein product  
30           of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis,

encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1842 of SEQ ID NO:52, b is an integer of 15 to 1856, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 43

The translation product of this gene shares sequence homology with a phosphotyrosine-independent ligand for the lck SH2 domain which is thought to be important in signal transduction related to phosphotyrosine-independent ligand for the lck SH2 domain, which may implicate this protein as playing an essential role in

regulating key cellular processes such as cellular division, and potentially in male fertility. (See Genbank Accession No. gi|1184951.)

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 5 ESSGQARTLADPGPGWPRQQGMCFGSLTGLSTTPHGFLT VSAEADPRLIESLS  
QMLSMGFSDEGGWLTRLLQTKNYDIGAALDTIQYSKH (SEQ ID NO:606),  
YSMVYTYHIFFIHSLLDGQLGWFHIFAIVSCAAPDIIFNSFAFSTYISKSCSFYLQ  
NVSCIHSSLSIFNLFQCPIISCMEECNNWLTGLFLHFKIKRCR (SEQ ID  
NO:607),  
10 LSPSPRCCPWASLMKAAGSPGSCRPTMTSERLWTPSSIQSIPRRCDHFCPPLL  
RAPLLSHSCVKLA (SEQ ID NO:608),  
GWPRQQGMCFGSLTGLSTTPHGFLT VSAEADPRL (SEQ ID NO:609),  
LGWFHIFAIVSCAAPDIIFNSFAFSTYISKSCS (SEQ ID NO:610),  
SLSIFNLFQCPIISCMEECNNWLTG (SEQ ID NO:611), and/or  
15 LMKAAGSPGSCRPTMTSERLWTPSSIQSI (SEQ ID NO:612). Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
20 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

It is likely that this gene is a new member of a family of phosphotyrosine-independent ligands for the lck SH2 domains,

- 25 This gene is expressed primarily in the placenta, and to a lesser extent, in  
endothelial cells and neutrophils.

- Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
30 reproductive, cardiovascular, immune, and infectious diseases. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s).

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For a number of disorders of the above tissues or cells, particularly of the cardiovascular, reproductive, and immune system, and infectious diseases, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, cardiovascular, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 291 as residues: Ile-93 to Arg-98.

The tissue distribution in placenta and endothelial tissues, combined with the homology to a phosphotyrosine-independent ligand for the lck SH2 domain indicates that the protein product of this gene is useful for diagnosis and treatment of cardiovascular, reproductive, and immune system diseases, as well as infectious diseases. Moreover, the polypeptide of this gene may be able to modulate T or B cell development and/or T or B cell activation (e.g. by modulation of Lck activity). It may also be capable of modulating degradation of cellular proteins (e.g. cell cycle regulatory proteins stimulating expression of cell cycle dependent kinase inhibitors and arresting cell cycle progression at specific boundaries to thereby modulate cell proliferation). p62 acts to boost B cell response and may be used to treat disorders where this is beneficial, e.g. infections by pathogenic microorganisms, e.g. bacteria, viruses and protozoans. p62 can be used to expand T cell populations for treating infectious diseases or cancer, e.g. the resulting cells may be transduced to render them resistant to HIV infection. Inhibitors of p62 can be used to reduce B or T cell responses and may be used to treat a variety of autoimmune diseases, e.g. diabetes mellitus, arthritis, multiple sclerosis allergic reactions, Crohn's diseases etc. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1544 of SEQ ID NO:53, b is an integer of 15 to 1558, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 44**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequences:

15 SSSSPRRPRELLGSLKTPLVRPHSAPLDLPGSFCXHTADPMGALHTRFWGRQT  
WIHRKLRLHGTSRLASKXGIQFLRNPSKTHTPRDAAFRDPGQTPDPQSLQAPS  
PSKCSAPNRATSVWSLKPRLLYKHRPSSDKTPPPGRQAPLLFFSAG (SEQ ID  
NO:613), and/or FLRNPSKTHTPRDAAFRDPGQTPDPQSLQA (SEQ ID NO:614).

Moreover, fragments and variants of these polypeptides (such as, for example,  
20 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides ) are encompassed by the invention. Antibodies that  
bind polypeptides of the invention are also encompassed by the invention.

25 Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the fetal brain, cerebellum, and to a lesser extent, in the placenta.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
30 and for diagnosis of diseases and conditions which include, but are not limited to,  
neural, developmental, or reproductive disorders, particularly cancers. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neuronal cell related disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, reproductive, vascular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 292 as residues: Thr-20 to Gly-28.

The tissue distribution in fetal brain, combined with the homology to proline-rich protein genes indicates that the protein product of this gene is useful for diagnosis and treatment of neuronal cell related disorders. Similarly, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Moreover, expression within fetal tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions

involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 934 of SEQ ID NO:54, b is an integer of 15 to 948, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 45**

The translation product of this gene shares sequence homology with precerebellin of human, which is thought to be important in synaptic physiology. (See Genbank Accession No. gi|180251.) The cerebellum contains a hexadecapeptide, termed cerebellin, that is conserved in sequence from human to chicken. Three independent, overlapping cDNA genes have been isolated from a human cerebellum cDNA library that encode the cerebellin sequence. The longest gene codes for a protein of 193 amino acids that we term precerebellin. This protein has a significant similarity (31.3% identity, 52.2% similarity) to the globular (non-collagen-like) region of the B chain of human complement component C1q. The region of relatedness extends over approximately 145 amino acids located in the carboxyl terminus of both proteins. Unlike C1q B chain, no collagen-like motifs are present in the amino-terminal regions of precerebellin. The amino terminus of precerebellin contains three possible N-linked glycosylation sites. Although

hydrophobic amino acids are clustered at the amino terminus, they do not conform to the classical signal-peptide motif, and no other obvious membrane-spanning domains are predicted from the cDNA sequence. The cDNA predicts that the cerebellin peptide is flanked by Val-Arg and Glu-Pro residues. Therefore, cerebellin is not liberated from precerebellin by the classical dibasic amino acid proteolytic-cleavage mechanism seen in many neuropeptide precursors. In Northern (RNA) blots, precerebellin transcripts, with four distinct sizes (1.8, 2.3, 2.7, and 3.0 kilobases), are abundant in cerebellum. These transcripts are present at either very low or undetectable levels in other brain areas and extraneural structures. A similar pattern of cerebellin precursor transcripts are seen in rat, mouse, and human cerebellum. Furthermore, a partial genomic fragment from mouse shows the same bands in Northern blots as the human cDNA gene. During rat development, precerebellin transcripts mirror the level of cerebellin peptide. Low levels of precerebellin mRNA are seen at birth. Levels increase modestly from postpartum day 1 to 8, then increase more dramatically between day 5 and 15, and eventually reach peak values between day 21 and 56. It has been observed that cerebellin-like immunoreactivity is associated with Purkinje cell postsynaptic structures. Thus, it is likely that this gene also have synaptic activity. Northern analysis showed a brain-specific 2.4kb message. This is consistent with the current insert size we have, suggesting our gene is full-length and is brain-specific.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

QEGSEPVLLGECLVVCEPGRAAAGGPGGAALGEAPPGRVAFXAVRSHHHEP  
 AGETGNGTSGAIYFDQVLVNEG GGFDRASGSFVAPVRGVYSFRFHVVKVYN  
 RQTVQVSLMLNTWPVISAFANDPDVTREAAATSSVLLPLDPGDRVSLRLRRGX  
 STGW (SEQ ID NO:615), GETGNGTSGAIYFDQVLVNEG GGFDRASGSFVAPV  
 (SEQ ID NO:616), NDPDVTREAAATSSVLLPLDPGDRVS (SEQ ID NO:617),  
 FHVVKVYNRQT (SEQ ID NO:618), IYFDQVLVN (SEQ ID NO:619),  
 ESRERSGNRRGAEDRGTCGLQSPSA (SEQ ID NO:620),  
 EMPQFYFFLKLGLCLAQVPMQRGGIGARGSXXPAXAVXGAREGRRKLSGAGF  
 LCLKDLGPSEREDEEARET (SEQ ID NO:621),  
 MPQFYFFLKLGLCLAQVPMQRGGIGARG (SEQ ID NO:622),



QATCSASGSPGQFGGCTPSPHGTGSCRHPGQGLRRSQRPQSHRPRSPGPGRS  
 RWPHWCHCRFPLLAHGGGFGPQQMPLAQGVPLPGLLPRAPLQQLGQAHRRP  
 GTPPPAGRALTPPGPTRPPGPEAPEPRAARDCVGDLDVASVAWLPTWLRGSAT  
 HKCPGLLPLFCFRSSPWILTAGTLIVCPL (SEQ ID NO:623),

5 GCTPSPHGTGSCRHPGQGLRRSQRP (SEQ ID NO:624),  
 SRWPHWCHCRFPLLAHGGGFGPQQMP (SEQ ID NO:625),  
 DCVGDLDVASVAWLPTWLRGSATHKCPGL (SEQ ID NO:626),  
 DDRPRVQHQAHLDLAVVHLHHMEPEAVDTPDRGYEGARGPVKATALVHQ  
 DLVEVDGPTGAIAGFPCWLMVVASDRXKCHSPRGCLSQGCSPGPPCSSSARL  
 10 TDHQALPLQQDGL (SEQ ID NO:627),

YEGARGPVKATALVHQDLVEVDGPTGAIAGF (SEQ ID NO:628),  
 MAPLVPLPVSPAGSWWLRTAXNATRPGGASPRAPPGPAAARPGSQTTT  
 HSPSSRTGSDPSWAHPAPRARSTRTKGSPGLCRGPGSQCGLAPNMAEGLCNP  
 QVPRSSAPLLFLLSLDSHRRHPDSLPSLGSNLPLSIPVSQQLCPASHSYSCCHCS

15 S (SEQ ID NO:629), SSRTGSDPSWAHPAPRARSTRTKGSPGLC (SEQ ID  
 NO:630), and/or RRHPDSLPSLGSNLPLSIPVSQQLCPAS (SEQ ID NO:631).

Moreover, fragments and variants of these polypeptides (such as, for example,  
 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
 20 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides ) are encompassed by the invention. Antibodies that  
 bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

25 This gene is expressed primarily in cerebellum and infant brain. By Northern  
 analysis, a single transcript of 2.4 kb was observed in brain tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 neural and developmental disorders, particularly neuronal cell signal transduction,  
 30 synaptic physiology, or proliferative conditions such as cancer. Similarly,  
 polypeptides and antibodies directed to these polypeptides are useful in providing  
 immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the neuronal cell signal transduction and synaptic physiology expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in cerebellum and infant brain, combined with the homology to the conserved precerebellin gene or gene family indicates that the protein product of this gene is useful for diagnosis and treatment of neuronal cell related disorders. Furthermore, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:55 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 976 of SEQ ID NO:55, b is an integer of 15 to 990, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 46

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
 STHASGPPAPERLCLPERGTAPWGRRANDAA (SEQ ID NO:632),  
 VRRWWLRTMGAAAHCTPEQRRPRRPATILGMDTQNILHTRLSLCSLSWVSL  
 ASSFXXLAXRRKAIVVQQKQSKISKKKKVEKXXLNSVNNENSDTVGQIVHYI  
 MKNEANADVLKAMVADNSLYDPESPVTPSTPGSPPVSPGLCHQGGRQGSTS  
 VAIICIRWAVXSRGMCVIGVGTSGGTL (SEQ ID NO:633), and/or  
 IMKNEANADVLKAMVADNSLYDPESPVTP (SEQ ID NO:634). Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

This gene is expressed in fetal liver and spleen, and to a lesser extent in bone marrow, umbilical vein, and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the immune system, particularly hematopoiesis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological

probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoiesis and immune disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 294 as residues: Asp-30 to Glu-57.

The tissue distribution in fetal liver/spleen and bone marrow indicates that the protein product of this gene is useful for diagnosis and treatment of hematopoietic and immune disorders. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:56 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

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### FEATURES OF PROTEIN ENCODED BY GENE NO: 47

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This gene is expressed primarily in human cardiomyopathy tissue, and to a lesser extent, in T helper cells, fetal brain and synovial sarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cardiovascular, immune, or developmental disorders, particularly cardiomyopathy which occur secondary to viral infections. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cardiovascular, neural, developmental, skeletal, immune cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 295 as residues: Trp-20 to Cys-26.

The tissue distribution in cardiomyopathy tissue, combined with the homology to a viral 12 kD nucleic acid binding protein indicates that the protein product of this gene is useful for diagnosis and intervention of cardiomyopathy, including those caused by ischemic, hypertensive, congenital, valvular, or pericardial abnormalities. The gene expression pattern may be the consequence or the cause for these conditions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:57 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1038 of SEQ ID NO:57, b is an

integer of 15 to 1052, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 48

The translation product of this gene shares sequence homology with tumor necrosis factor related gene product, which is thought to be important in tumor necrosis, bacterial and viral infection, immune diseases and immunoreactions.

10 In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

KMNSIPWQIPKITPXLDANLVIVECKPLWFCIGTIKQLKLWNQVFMGFKSMFF  
RIGKLNLYLFTIPYCYLFIDNILGIFYSILGAQGIKYNFYIQRIFTCLLNLNLKIHSN  
LA (SEQ ID NO:639), LWFCIGTIKQLKLWNQVFMGFKSMFFR (SEQ ID

15 NO:640), YSILGAQGIKYNFYIQRIFTCLLNLN (SEQ ID NO:641), and/or  
TFKLVRFL (SEQ ID NO:642). Moreover, fragments and variants of these  
polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
20 conditions, to the polynucleotide encoding these polypeptides ) are encompassed by  
the invention. Antibodies that bind polypeptides of the invention are also  
encompassed by the invention. Polynucleotides encoding these polypeptides are also  
encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
25 10. Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 10.

This gene is expressed primarily in colon, and to a lesser extent, in ovarian  
and breast cancers.

Polynucleotides and polypeptides of the invention are useful as reagents for  
30 differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
gastrointestinal, reproductive, colon, ovarian, breast disorders, particularly cancers.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the colon, ovary and breast, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, reproductive, colon, ovarian, breast, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, breast milk, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in colon tissue, combined with the homology to tumor necrosis factors indicates that the protein product of this gene is useful for the intervention of cancers of the colon, ovary and breast, particularly because TNF family members are known to be involved in the tumor development. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:58 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 800 of SEQ ID NO:58, b is an integer of 15 to 814, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 49**

The translation product of this gene shares sequence homology with mucins, such as epithelial mucin, which are thought to be important in extracellular matrix



functions such as protection, lubrication and cell adhesion, which are important in a variety of functions, particularly immune chemotaxis and infiltration (See for example Genbank Accession No. R68002).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

PRSRPALRPGRQRPPSHSATSGVLRPRKKPDP (SEQ ID NO:643),  
 RKSFAPVLTWNAIQAGRGRVLCYTRPPPASSFSALVPDGNRMEGLRTYFL  
 NAFDPGTDYLYLFPFSFTVTFQHCLTVRWAFESLQVPQNRPERWASHPLPTH  
 XPAYLPDNQVXMSASG (SEQ ID NO:644),  
 GNRMEGLRTYFLNAFDPGTDYLYLF (SEQ ID NO:645), and/or  
 FQHCLTVRWAFESLQVPQNRPERWASHPLP (SEQ ID NO:646). Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

Moreover, this gene maps to chromosome 22q11.2-qter, and therefore, can be  
 used as a marker in linkage analysis for chromosome 22.

This gene is expressed primarily in corpus colosum.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tumors, especially of the corpus colosum, as well as metastatic lesions, autoimmune conditions, and integumentary disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the corpus colosum and other solid tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., integumentary, autoimmune, neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine,

synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution in corpus colosum, combined with the homology to mucins indicates that the protein product of this gene is useful for serum tumor markers or immunotherapy targets because tumor cells have greatly elevated levels of mucin expression and shed the molecules into the epithelial tissues. Moreover, the protein product of this gene is useful for the treatment, diagnosis, and/or prevention of

10 various skin disorders including congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder,

15 psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox,

20 molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, Athlete's foot, and ringworm). Moreover, the protein product of this gene may also be useful for the treatment or diagnosis of various connective tissue disorders such as arthritis, trauma, tendonitis, chondromalacia and inflammation, autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as

25 dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:59 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

5 formula of a-b, where a is any integer between 1 to 1201 of SEQ ID NO:59, b is an integer of 15 to 1215, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 50

This gene is expressed primarily in CD34 depleted buffy coat cord blood and primary dendritic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for

15 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic disorders and immunological disorders, particularly those related to developmental or reproductive conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for

20 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid,

25 serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 depleted buffy coat cord blood and primary

30 dendritic cells indicates that the protein product of this gene is useful for the diagnosis and treatment of hematopoietic and immune disorders. Secreted or cell surface proteins in the above tissue distribution often are involved in cell activation (e.g.

cytokines) or molecules involved in cell surface activation. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages.

5 The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells  
10 and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:60 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
20 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 464 of SEQ ID NO:60, b is an integer of 15 to 478, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.

## 25 **FEATURES OF PROTEIN ENCODED BY GENE NO: 51**

The translation product of this gene shares sequence homology with Interferon induced 1-8 gene encoded polypeptide, which is thought to be important in binding to  
30 retroviral rev responsive elements and may be beneficial in the development of novel inhibitors of host-viral interactions leading to effective viral vaccines.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MTLITPSXKLTFXKGNKSWSSRACSTLVDP (SEQ ID NO:647),

FLFLHAVDPWPSNG (SEQ ID NO:648),

5 WSCQSGVFLVFTGCSVLCQMLSGAVVWRRSAPEDSAVWQASINKPRGKGR  
HGIKGENTSV (SEQ ID NO:649), and/or LVFTGC

SVLCQMLSGAVVWRRSAPEDSAVWQASI (SEQ ID NO:650). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or

10 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in CD34 positive cells and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, viral infection, such as AIDS, and other immune or hematopoietic disorders.

20 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and  
25 cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

30 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 299 as residues: Gln-51 to Trp-62.

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The tissue distribution in neutrophils and CD34 positive cells, combined with the homology to interferon induced gene 1-8 indicates that the protein product of this gene is useful for the intervention of retroviral infection including HIV. The factor may be involved in viral stability or viral entry into the cells. Alternatively, the virus/factor complex may elicit the cellular immune reaction and could possibly play a beneficial role in the development of effective inhibitors of host-viral interactions, such as exists for novel viral vaccines. Moreover, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:61 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 604 of SEQ ID NO:61, b is an

integer of 15 to 618, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 52

This gene shares sequence homology to immunoglobulin lambda chain (See Genbank Accession No. 2865484). Therefore it is likely that this gene has activity similar to an immunoglobulin lambda chain and may play a beneficial role in the development of effective immunotherapy-based toxins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

GHPSPALSIAPSDGSQLPCDEVYPYGEAHVTRYCKPLTNSHLETEAQSSSL  
(SEQ ID NO:651),

NNKHYSFCGSGFCPVYLGFTGLASHQAVKVLVAVIIPRQDRERICLQAQV  
GRIHLRGCWTGPPFLDGYWSEAFYNTLSRGPLHRAPHHMATGFHQREQWKE  
QEKGDQGRHRSLLVASPQKRKYFCCILXVRSESLGPGVEFYXGVNGRR (SEQ  
ID NO:652), ERICLQAQVGRIHLRGCWTGPPFLDGYWSEAF (SEQ ID NO:653),  
SDGSQLPCDEVYPYGEAHVTRYCKKPL (SEQ ID NO:654), and/or

HQREQWKEQEKGDQGRHRSLLVASPQK (SEQ ID NO:655). Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Hodgkin's lymphoma.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
Hodgkin's lymphoma and other immune or hematopoietic disorders. Similarly,

polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the

10 disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 300 as residues: Pro-27 to Thr-32.

The tissue distribution in Hodgkin's lymphoma, combined with the sequence homology to immunoglobulin lambda chain protein indicates that the protein product

15 of this gene is useful for the diagnosis of Hodgkin's lymphoma, since the elevated expression and secretion by the tumor mass may be indicative of tumors of this type. Additionally the gene product may be used as a target in the immunotherapy of the cancer. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an

20 agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:62 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

30 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 737 of SEQ ID NO:62, b is an



integer of 15 to 751, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 53

This gene has extensive homology to cDNA for Homo sapiens mRNA for the ISLR gene(See Genbank Accession No. AB003184). This protein is considered to be a new member of the Ig superfamily and contains a leucine-rich repeat (LRR) with conserved flanking sequences and a C2-type immunoglobulin (Ig)-like domain. These domains are important for protein-protein interaction or cell adhesion, and therefore it is possible that the novel protein ISLR may also interact with other proteins or cells. The ISLR gene was mapped on human chromosome 15q23-q24 by fluorescence in situ hybridization (See Medline Article No. 97468140). Homology to the ISLR gene has been confirmed by another independent group as well (See Genbank Accession No. Hs.102171).

This gene is expressed in a number of tissues including human retina, heart, skeletal muscle, prostate, ovary, small intestine, thyroid, adrenal cortex, testis, stomach, spinal cord, fetal lung and fetal kidney tissues, colon, tonsil and stomach cancer, and to a lesser extent in endometrial stromal cells treated with estradiol, breast tissue, synovium, lymphoma, and number of other tumors.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tumors of colon, ovary, breast, and integumentary or immune origins. However, due to the wide range of expression in various tissues, protein may play a vital role in the development of cancer in other tissues as well, not just those mentioned above. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the colon, ovary and breast, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune,

integumentary, reproductive, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, breast milk, seminal fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. Additionally, this gene maps to chromosome 15q23-q24, and therefore, can be used as a marker in linkage analysis for chromosome 15.

The tissue distribution in tumors of colon, ovary, and breast origins indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds); stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction, etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:63 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 766 of SEQ ID NO:63, b is an integer of 15 to 780, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 54

The gene has homology to a multidrug resistance gene 1 (See Genbank Accession No. P06795).

Preferred polynucleotide fragments comprise the following sequence:

gcttcgtgtccaaccctcttgcccttcgcctgtgtgcctggagccagtcaccacgctcgcgttcctcctgtagtgctcaca  
 ggtcccagcaccgatggcattccctttgccctgagctcgcagcgggtccctttgtgcttcctcccctcaggtagcctctctc  
 cccctgggccactcccgggggtgagggggtacccttcccagtggtttttattcctgtggggctcaccccaaagtattaaaa  
 gtagctttgtaa (SEQ ID NO:656),

gcttcgtgtccaaccctcttgcccttcgcctgtgtgcctggagccagtcaccacgctcgcgttcctcctgtagtgctcaca  
 ggtcccagcaccgatggcattccctttgccctgagctcgcagcgggtccctttgtgcttcctcccctcaggtagcctctctc  
 cccctgggccactcccgggggtgagggggtacccttcccagtggtttttattcctgtggggctcaccccaaagtattaaaa  
 gtagctttgtaa (SEQ ID NO:657),

gcttcgtgtccaaccctcttgcccttcgcctgtgtgcctggagccagtcaccacgctcgcgttcctcctgtagtgctcaca  
 ggtcccagcaccgatggcattccctttgccctgagctcgcagcgggtccctttgtgcttcctcccctcaggtagcctctctc  
 cccctgggccactcccgggggtgagggggtacccttcccagtggtttttattcctgtggggctcaccccaaagtattaaaa  
 gtagctttgtaa (SEQ ID NO:658). Also preferred are polypeptides comprising one or

more of the fragments encoded by these polynucleotide fragments.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

FRINRLTIGXAVAMTRGNQRELARQKNMKKQSDSVKGKRRDDGLSAAARK  
 QRDSEI (SEQ ID NO:659), AVAMTRGNQRELARQKNMKKQSDSVKGKR (SEQ  
 ID NO:660),  
 KSRATRLRESAEMTGFLPPASRGTRRSCSRSRKRQTRRRRNPSFVASCPTLL

PFACVPGASPTTLAFPPVVLTPSTDGIPFALSQRVPFVLPSPQVASLPLGHSR  
 G (SEQ ID NO:661), LRESAEMTGFLPPASRGTRRSCSRS (SEQ ID NO:662),  
 and/or VVLTGPSTDGIPFALSQRVPFVLPSPQVA (SEQ ID NO:663). Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 5 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 10 these polypeptides are also encompassed by the invention.

This gene is expressed primarily in lung, esophagus, leukemia (Jurkat cells),  
 breast cancers and to a lesser extent, in macrophages treated with GM-CSF fetal  
 tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 15 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 immune, developmental, or pulmonary disorders, particularly cancers. Similarly,  
 polypeptides and antibodies directed to these polypeptides are useful in providing  
 immunological probes for differential identification of the tissue(s) or cell type(s).

20 For a number of disorders of the above tissues or cells, particularly of the solid  
 tumors, lung and leukemia, expression of this gene at significantly higher or lower  
 levels may be routinely detected in certain tissues or cell types (e.g., immune,  
 developmental, pulmonary, and cancerous and wounded tissues) or bodily fluids (e.g.,  
 lymph, pulmonary surfactant and sputum, amniotic fluid, serum, plasma, urine,  
 25 synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
 individual having such a disorder, relative to the standard gene expression level, i.e.,  
 the expression level in healthy tissue or bodily fluid from an individual not having the  
 disorder. Furthermore, due to the high expression level in lung tissue and the  
 proposed function of the multidrug resistance protein 1 gene as the efflux pump  
 30 responsible for low-drug accumulation in multidrug-resistant cells, protein as well  
 mutants thereof, may also be beneficial as a target for gene therapy, particularly for  
 the chronic patient.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 302 as residues: Met-1 to Lys-16.

The tissue distribution cancers and fetal tissues indicates that the protein product of this gene is useful for the detection of cells in active proliferation, such as cancers. The gene products may be used for cancer markers or immunotherapy target. Similarly, the secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds); stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction, etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:64 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 574 of SEQ ID NO:64, b is an

integer of 15 to 588, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 55

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

LLSTSHLLTQSYFNRSHSFAWKNAHCILQSENNELQNSVYIYVCIYVHF

10 ICTFLCDI (SEQ ID NO:664), and/or KRSHSFAWKNAHCILQSENNELQNSVYIY VCI (SEQ ID NO:665). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
15 polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on the X  
20 chromosome. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for the X chromosome.

This gene is expressed primarily in the brain, and to a lesser extent, in the developing embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for  
25 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disease states and developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).  
30 For a number of disorders, including X-linked disorders, of the above tissues or cells, particularly of the neurological, developmental systems, and cardiovascular system, expression of this gene at significantly higher or lower levels may be routinely

detected in certain tissues or cell types (e.g., neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neural tissue indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Klinefelter's, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually- or X-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:65 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 931 of SEQ ID NO:65, b is an integer of 15 to 945, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 56

The translation product of this gene shares sequence homology with paxillin, which is thought to be important in mediating signal transduction from growth factor

receptors to the cytoskeleton. Moreover, in normal hematopoietic cells and myeloid cell lines, tyrosine phosphorylation of paxillin has been shown to be rapidly and transiently induced by interleukin-3 and several other hematopoietic growth factors. The predicted structure of paxillin implicates this molecule in protein-protein interactions involved in signal transduction from growth factor receptors and the BCR/ABL oncogene fusion protein to the cytoskeleton.

Preferred polynucleotide fragments comprise the following sequence:

tggtcactgtcttacaatcactgtgtggaatcatgataccacttttagctctttgcatcttccttcagtgtattttgttttcaaga  
ggaagtagattttaactggacaactttgagtactgacatcattgataaataaactggcttggtgttcaa (SEQ ID

NO:666). Also preferred are polypeptide fragments encoded by these polynucleotide fragments.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

LDELMAHLTEMQAKVAVRADAGKKHLPDKQDHKASLDSMLGGLEQELQDL  
GIATVPKGHCASCQKPIAGKVIHALGQSWHPEHFVCTHCKEEIGSSPFFERSGL  
XYCPNDYHQLFSPRCAYCAAPILDKVLTAMNQTWHPHFCSHCGEVFGAE  
GFHEKDKKPYCRKDFLAMFSPKCGGCNRPVLENYLSAMDTVWHPECFVCG  
DCFTSFSTGSFFELDGRPFCELHYHHRRGTLCHGCGQPITGRCISAMGYKFHP  
EHFVCAFCLTQLSKGIFREQNDKTYCQPCFNKLF (SEQ ID NO:667),

KASLDSMLGGLEQELQDLGIATVPKGHCASCQKPIAGKVIHAL (SEQ ID  
NO:668),

CPNDYHQLFSPRCAYCAAPILDKVLTAMNQTWHPHFCSHCGEVFGAEG  
(SEQ ID NO:669),

DKKPYCRKDFLAMFSPKCGGCNRPVLENYLSAMDTVWHPECFVCGDCFTSF  
STGSFFELDGRPFCEL (SEQ ID NO:670),

CGQPITGRCISAMGYKFHPHFVCAFCLTQLSKGIFREQNDKTYCQ (SEQ ID  
NO:671),

HKSLAGAXVYTTNIQELNVYSEAQEPKESPPPSKTSAAAQLDELMAHLTEMQ  
AKVAVRADAGKKHLPDKQDHKASLDSMLGGLEQELQDLGIATVPKGHCAS  
CQKPIAGKVIHALGQSWHPEHFVCTHCKEEIGSSPFFERSGLXYCPNDYHQLF  
SPRCAYCAAPILDKVLTAMNQTWHPHFCSHCGEVFGAEGFHEKDKKPYC  
RKDFLAMFSPKCGGCNRPVLENYLSAMDTVWHPECFVCGDCFTSFSTGSFFE



LDGRPFCELYHHRRGTLCHGCGQPITGRCISAMGYKFHPEHFVCAFLTQLS  
 KGIFREQNDKTYCQPCFNKLFPL (SEQ ID NO:672),  
 NVYSEAQEPKESPPPSKTSAAA (SEQ ID NO:673),  
 DSMLGGLEQELQDLGIATVPKGHCAS (SEQ ID NO:674),  
 5 YLSAMDTVWHPECFVCGDCFTSFSTG (SEQ ID NO:675),  
 RCISAMGYKFHPEHFVCAFLTQLSK (SEQ ID NO:676),  
 PTRPVLFSTCQSCSSRPVRQEHLGCRTMEELDALLEELERSTLQDSDEYSNP  
 APLPLDQHSRKETNLDETSEILSIQDNTSPLPAXSCILPISRSSMSTVKPKSQRN  
 HHHLLKRQQLLSWMSSWLT (SEQ ID NO:677),

10 PVRQEHLGCRTMEELDALLEELERSTLQ (SEQ ID NO:678),  
 SCILPISRSSMSTVKPKSQRN (SEQ ID NO:679), WHPEHFVCTHC (SEQ ID  
 NO:680), LFSPRC (SEQ ID NO:681), PILDKV (SEQ ID NO:682), TWHPEHFF  
 (SEQ ID NO:683), EGFHEKD (SEQ ID NO:684), KFHPEHFVCAFL (SEQ ID  
 NO:685), PITGRCI (SEQ ID NO:686), and/or HPEHFVC (SEQ ID NO:687).

15 Moreover, fragments and variants of these polypeptides (such as, for example,  
 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides ) are encompassed by the invention. Antibodies that  
 20 bind polypeptides of the invention are also encompassed by the invention.  
 Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
 11. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 11.

25 This gene is expressed primarily in brain, and to a lesser extent in the  
 developing embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 30 neurological disease states and developmental abnormalities. Similarly, polypeptides  
 and antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of

disorders of the above tissues or cells, particularly of the immune and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g.,

5 lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain, combined with the homology to the conserved  
10 paxillin gene, indicates that the protein product of this gene is useful for the treatment and or detection of disease states associated with abnormal signal transduction in brain and/or the developing embryo. This would include treatment or detection of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia,  
15 paranoia, obsessive compulsive disorder and panic disorder and also in the treatment and or detection of embryonic development defects. Moreover, expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders.

20 Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:66 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
30 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1852 of SEQ ID NO:66, b is an

integer of 15 to 1866, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 57

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

RIYCS EDTFSPXAESGVSWQSSVSQLYQDYE (SEQ ID NO:688). Moreover,

10 fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of

15 the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in fetal spleen, brain, and to a lesser extent, in six week old embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for

20 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders, neurological disorders, and developmental abnormalities.

Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell

25 type(s). For a number of disorders of the above tissues or cells, particularly of the immune and developmental systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or

30 another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 305 as residues: Arg-28 to Gly-34.

The tissue distribution in fetal spleen indicates that the protein product of this gene is useful for the treatment/detection of immune disorders such as arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. In addition the expression of this gene in the early embryo, indicates a key role in embryo development, and hence the gene or gene product could be used in the treatment and or detection of embryonic developmental defects. This would include treatment or detection of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder and also in the treatment and or detection of embryonic development defects. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:67 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1138 of SEQ ID NO:67, b is an integer of 15 to 1152, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 58**

The translation product of this gene shares sequence homology with the gene disrupted in the neurodegenerative disease dentatorubal-pallidoluysian atrophy. Moreover, the translation product of this gene also shares homology with the

GRASP65 protein, a protein involved in the stacking of Golgi cisternae (See Genbank Accession No. AF015264).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 5 MGSSQSVEIPGGGTEGYHVLRVQENSPGHRAGLEPFFDFIVSINGSRLNKDND  
TLKDLLKXNVEKPVKMLIYSSKTLELRETSVTPSNLWGGQGLLGVSIRFCSFD  
GANENVWHVLEVESNSPAALAGLRPHSDYIIGADTVMNESEDLFSLIETHEAK  
PLKLYVYNTD TDNCREVIITPNSAWGGEGSLGCGIGYGYLHRIPTRPFEEGKKI  
SLPGQMAGTPITPLKDGFTFVQLSSVNPPSLSPPGTTGIEQSLTGLSISSTPPAVS
- 10 SVLSTGVPTVPLLPPQVNQSLTSVPPMNPATTLPLGLMPLPAGLPNLPNLPNLP  
PAPHIMPGVGLPELVNPGLPPLPSMPPRNLPGLAPLPSEFLPSFPLVPESSSAA  
SSGELLSSLPPTSNAPSDPATTTAKADAASSLTVDVTPPTAKAPTTVEDRVGD  
STPVSEKPVSAAVDANASESP (SEQ ID NO:689),  
SVEIPGGGTEGYHVLRVQENSPGHRAGLEPFFDFIVSINGSRLNKDNDTLKDL
- 15 LKXNVEKPVKMLIYSSKTLELRETSVTPSNLWGGQGLLGVSIRFCSFDGANEN  
VWH (SEQ ID NO:690),  
ESNSPAALAGLRPHSDYIIGADTVMNESEDLFSLIETHEAKPLKLYVYNTD TD  
NCREVIITPNSAWGGEGSLGCGIGYGYLHRIPTRPFEEGKKISLPGQMAGTPIT  
PLKDGFTFVQLSSVNPPSLSPPGTTGIEQSLTGLSISS (SEQ ID NO:691),
- 20 ESNSPAALAGLRPHSDYIIGADTVMNESEDLFSLIETHEAKPLKLYVYNTD TD  
NCREVIITPNSAWGGEGSLGCGIGYGYLHRIPTRPFEEGKKISLPGQMAGTPIT  
PLKDGFTFVQLSSVNPPSLSPPGTTGIEQSLTGLSISS (SEQ ID NO:692)  
RIPTRPFEEGKKISLPGQMAGTPITPLKDGFTFVQLSSVNPPSLSPPGTTGIEQSL  
TGLSISSTPPAVSSVLSTGVPTVPLLPPQVNQSLTSVPPMNPATTLPLGLMPLPA
- 25 GLPNLPNLPNLPAPHIMPGVGLPELVNPGLPPLPSMPPRN (SEQ ID NO:693),  
PGLPPLPSMPPRNLPGLAPLPSEFLPSFPLVPESSSAAASSGELLSSLPPTSNAPS  
DPATTTAKADAASSLTVDVTPPTAKAPTTVEDRVGDSTPVSEKPVSAAVDAN  
(SEQ ID NO:694), AWGGEGSLGCGIGYGYLHRIPT (SEQ ID NO:695),  
SPAALAGLRP (SEQ ID NO:696), and/or WGGQGLLG (SEQ ID NO:697).
- 30 Moreover, fragments and variants of these polypeptides (such as, for example,  
fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the

polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 5           The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in prostate cancer, and to a lesser extent, in the pineal glands and in fetal lung.

- 10           Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological, endocrine, reproductive, pulmonary, developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
15           providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous, pulmonary, and endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neurological, endocrine, reproductive, pulmonary, developmental, and cancerous and  
20           wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, pulmonary surfactant and sputum, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- 25           Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 306 as residues: Asn-9 to Leu-14.

- The abundance of this gene in the pineal gland and its homology to a gene disrupted in the neurodegenerative disease state Dentatorubral-pallidoluyisian atrophy indicates that this gene may be useful in the treatment and/or detection of other  
30           neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. Alternatively, the

abundance of this gene in fetal lung would suggest that misregulation of the expression of this protein product in the adult could lead to lymphoma or sarcoma formation, particularly in the lung; that it may also be involved in predisposition to certain pulmonary defects such as pulmonary edema and embolism, bronchitis and cystic fibrosis; and thus the gene or the gene product encoded by the gene could be used in the detection and/or treatment of these pulmonary disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:68 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2469 of SEQ ID NO:68, b is an integer of 15 to 2483, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 59**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

RNGALLDKNFFNANSHFPVKGERIRRR (SEQ ID NO:698). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in the developing embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental abnormalities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developmental system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developing, proliferating, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene primarily in the embryo indicates the gene plays a key role in embryo development, and that the gene or the protein encoded by the gene could be used in the treatment and or detection of developmental defects in the embryo or in infants. Similarly, the relatively specific expression of this gene product during embryogenesis indicates that it may be a key player in the proliferation, maintenance, and/or differentiation of various cell types during development. It may also act as a morphogen to control cell and tissue type specification. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.



Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:69 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 522 of SEQ ID NO:69, b is an integer of 15 to 536, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.

#### *FEATURES OF PROTEIN ENCODED BY GENE NO: 60*

This gene displays homology to nestin, an intermediate filament protein, the expression of which correlates with the proliferation of central nervous system progenitor cells and is useful in the identification of brain tumors.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

RGSGFGWTSFPRPLPTELTCPGFHRERAFPPDGRVRGVRGWGIRRGCRVWG  
VGACGCSPGSSWRGSAHRASGPADLPVACRXEGGADSPSLLPSPP (SEQ ID  
NO:699), AVWGVGACGCSPGSSWRGSAHRA (SEQ ID NO:700), YRP  
TMEKMKQVVTQTRWMPDAKRANRRHRRISGKIFAWNPLPKTRFSRLLKAV  
SENTKRPEPSRPPWMVSHSVEAS (SEQ ID NO:701), and/or  
FAWNPLPKTRFSRLLKAVSENTKRPEP (SEQ ID NO:702). Moreover, fragments  
and variants of these polypeptides (such as, for example, fragments as described  
herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%  
identical to these polypeptides and polypeptides encoded by the polynucleotide which  
hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in kidney, and to a lesser extent, in brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, renal disorders and neurodegenerative conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the excretory and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, urogenital, renal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID

NO: 308 as residues: Thr-130 to Asn-137.

The tissue distribution in brain and kidney, combined with the homology to the conserved nestin protein, indicates that the protein product of this gene is useful for the detection and/or treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, its abundance in kidney indicates that it is useful in the treatment and detection of acute renal failure and other disease states associated with the kidney, such as nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies directed

against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. .

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:70 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 560 of SEQ ID NO:70, b is an integer of 15 to 574, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.

## 15 FEATURES OF PROTEIN ENCODED BY GENE NO: 61

This gene shares homology with the latrophilin-related protein 1 precursor as well as the calcium-independent alpha-latrotoxin receptor. alpha-Latrotoxin, a black widow spider neurotoxin, can bind to high affinity receptors on the presynaptic plasma membrane and stimulate massive neurotransmitter release in the absence of Ca<sup>2+</sup>. Neurexins, previously isolated as alpha-latrotoxin receptors, require Ca<sup>2+</sup> for their interaction with the toxin and, thus, may not participate in the Ca<sup>2+</sup>-independent alpha-latrotoxin activity. However, latrophilin binds alpha-Latrotoxin with high affinity in the presence of various divalent cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ba<sup>2+</sup>, and Sr<sup>2+</sup>) as well as in EDTA. This presumably membrane-bound protein is localized to and differentially distributed among neuronal tissues, with about four times more latrophilin expressed in the cerebral cortex than in the cerebellum; subcellular fractionation showed that the protein is highly enriched in synaptosomal plasma membranes.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
IYKVFRHTAGLKPEVSCFENIRSCARXXXXXXXXXXXXXWIFGVLHVHVASV

VTAYLFTVSNAFQGMFIFLFLCVLSRKIQEEYYRLFKNVPCC (SEQ ID NO:703),

WIFGVLHVHVASVVTAYLFTVSNAFQGMFIFLFLCVLSRKIQEEYYRLFKNVPCC (SEQ ID NO:704), IYKVFRHTAGLKPEVSCFENIRSCAR (SEQ ID NO:705),

5 IYKVFRHTAGLKPEVSCFENIRSCARGALALLFLLGTTWIFGVLHVHVASVVTAYLFTVSNAFQG (SEQ ID NO:706), and/or

EVSCFENIRSCARGALALLFLLGTTWIFGVLH (SEQ ID NO:707). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 10 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 The translation product of this gene also shares sequence homology with CD 97, a seven transmembrane bound receptor (see Genbank Accession No. 2213659). The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

20 This gene is expressed primarily in infant brain and in endothelial cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological, vascular, and hematopoietic disorders. Similarly, polypeptides and 25 antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neurological and hematopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., vascular, neural, 30 hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene

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expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 309 as residues: Lys-13 to Leu-21.

5 The tissue distribution in infant brain genes suggest that the protein product may be useful in the detection and/or treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder, while its expression in hematopoietic cell  
10 types indicates that the gene could be important for the treatment or detection of immune or hematopoietic disorders including arthritis, asthma and immunodeficiency diseases. Moreover, the expression within endothelial tissue indicates that the protein product of this gene may show utility in the treatment and/or prevention of a variety of vascular disorders, which include, but are not limited to microvascular disease,  
15 atherosclerosis, stroke, embolism, and aneurysm. Furthermore, expression within infant tissue indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also  
20 be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
25 related to SEQ ID NO:71 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
30 formula of a-b, where a is any integer between 1 to 918 of SEQ ID NO:71, b is an integer of 15 to 932, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 62

5 In a specific embodiment, polypeptides of the invention, comprise or alternatively consist of, one or more of the following amino acid sequences:  
 TTILRTCTIVCFYYWFNGVMVLLFFLDRNLLTFNQASIMPFSNTDFLHCLSFK  
 KKLMLLRYIFYVVLGTGPTLSLKGDENQIKNLFT (SEQ ID NO:708),  
 IVCFYWFNGVMVLLFFLDRNLL (SEQ ID NO:709), and/or

10 LLRYIFYVVLGTGPTLSLKGDENQI (SEQ ID NO:710). Polynucleotides encoding these polypeptides are also encompassed by the invention as are antibodies that bind one or more of these polypeptides. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
 15 and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

20 Also preferred are polypeptides, comprising or alternatively consisting of, the mature polypeptide which is predicted to consist of residues:  
 PTCYSRMRLSQEITRDFNLLQVSEPSEPCVRYLPRLYLDIHNYCVLDKLRDF  
 VASPPCWKVAQVDSLKDKARKLYTIMNSFCRRDLVFLDDCNALEYPIPVTT  
 VLPDRQR (SEQ ID NO:1245) of the foregoing sequence (SEQ ID NO:310), and  
 25 biologically active fragments of the mature polypeptide (e.g., fragments that induce hematopoiesis). Polynucleotides encoding these polypeptides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
 30 encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are

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also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Figures 5A-5B show the nucleotide (SEQ ID NO:72) and deduced amino acid sequence (SEQ ID NO:310) corresponding to this gene.

Figure 6 shows an analysis of the amino acid sequence (SEQ ID NO:310). Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings of the recited computer algorithms. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. Polypeptides comprising, or alternatively consisting of, domains defined by these graphs are contemplated by the present invention, as are polynucleotides encoding these polypeptides.

The data presented in Figure 6 are also represented in tabular form in Table 5. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 6, and Table 5: "Res": amino acid residue of SEQ ID NO:310 and Figures 5A-5B; "Position": position of the corresponding residue within SEQ ID NO:310 and Figures 5A-5B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consisting of, one or more of the following regions: alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-

forming regions and high antigenic index regions. The data representing the structural or functional attributes of the protein set forth in Figures 5A-5B and/or Table 5, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table 5 can be used to determine regions of the protein which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figures 5A-5B, but may, as shown in Table 5, be represented or identified by using tabular representations of the data presented in Figure 6. The DNA\*STAR computer algorithm used to generate Figure 6 (set on the original default parameters) was used to present the data in Figure 6 in a tabular format (See Table 5). The tabular format of the data in Figure 6 is used to easily determine specific boundaries of a preferred region.

The present invention is further directed to fragments of the polynucleotide sequences described herein. By a fragment of, for example, the polynucleotide sequence of a deposited cDNA or the nucleotide sequence shown in SEQ ID NO: 72, is intended polynucleotide fragments at least about 15nt, and more preferably at least about 20 nt, at least about 25nt, still more preferably at least about 30 nt, at least about 35nt, and even more preferably, at least about 40 nt in length, at least about 45nt in length, at least about 50nt in length, at least about 60nt in length, at least about 70nt in length, at least about 80nt in length, at least about 90nt in length, at least about 100nt in length, at least about 125nt in length, at least about 150nt in length, at least about 175nt in length, which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 200-500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the nucleotide sequence of a deposited cDNA or as shown in SEQ ID NO:72. By a fragment at least 20 nt in length, for example, is intended fragments



which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:72. In this context "about" includes the particularly recited size, an sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

- 5 Representative examples of polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to about 50, from about 51 to about 100, from about 101 to about 150, from about 151 to about 200, from about 201 to about 250, from about 251 to about 300, from about 301 to about 350, from about 351 to about 400, from about 401 to about 450, from about 451 to about 500, and from about 501 to about 550, and from about 551 to about 600, from about 601 to about 650, from about 651 to about 700, from about 701 to about 750, from about 751 to about 800, from about 801 to about 850, from about 851 to about 900, from about 901 to about 950, or from about 951 to about 985 of SEQ ID NO:72, or the complementary strand thereto, or the cDNA
- 10 contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In additional embodiments, the polynucleotides of the invention encode functional attributes of the corresponding protein.
- 15 Preferred polypeptide fragments of the invention comprise, or alternatively consist of, the secreted protein having a continuous series of deleted residues from the amino or the carboxyl terminus, or both. Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-136 where m is an integer from 2 to 136, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:310. More in particular, the invention provides polynucleotides encoding
- 20 polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: R-2 to R-136; T-3 to R-136; P-4 to R-136; G-5 to R-136; P-6 to R-136; L-7 to R-136; P-8 to R-136; V-9 to R-136; L-10 to R-136; L-11 to R-136; L-12 to R-136; L-13 to R-136; L-14 to R-136; A-15 to R-136; G-16 to R-136; A-17 to R-136; P-18 to R-136; A-19 to R-136; A-20 to R-136; R-21 to R-136; P-22 to R-136; T-23 to R-136; P-24 to R-136; P-25 to R-136; T-26 to R-136; C-27 to R-136; Y-28 to R-136; S-29 to R-136; R-30 to R-136; M-31 to R-136; R-32 to R-136; A-33 to R-136; L-34 to R-136; S-35 to R-136; Q-36 to R-136; E-37 to R-136; I-38 to R-136; T-39 to
- 25
- 30

R-136; R-40 to R-136; D-41 to R-136; F-42 to R-136; N-43 to R-136; L-44 to R-136; L-45 to R-136; Q-46 to R-136; V-47 to R-136; S-48 to R-136; E-49 to R-136; P-50 to R-136; S-51 to R-136; E-52 to R-136; P-53 to R-136; C-54 to R-136; V-55 to R-136; R-56 to R-136; Y-57 to R-136; L-58 to R-136; P-59 to R-136; R-60 to R-136; L-61 to R-136; Y-62 to R-136; L-63 to R-136; D-64 to R-136; I-65 to R-136; H-66 to R-136; N-67 to R-136; Y-68 to R-136; C-69 to R-136; V-70 to R-136; L-71 to R-136; D-72 to R-136; K-73 to R-136; L-74 to R-136; R-75 to R-136; D-76 to R-136; F-77 to R-136; V-78 to R-136; A-79 to R-136; S-80 to R-136; P-81 to R-136; P-82 to R-136; C-83 to R-136; W-84 to R-136; K-85 to R-136; V-86 to R-136; A-87 to R-136; Q-88 to R-136; V-89 to R-136; D-90 to R-136; S-91 to R-136; L-92 to R-136; K-93 to R-136; D-94 to R-136; K-95 to R-136; A-96 to R-136; R-97 to R-136; K-98 to R-136; L-99 to R-136; Y-100 to R-136; T-101 to R-136; I-102 to R-136; M-103 to R-136; N-104 to R-136; S-105 to R-136; F-106 to R-136; C-107 to R-136; R-108 to R-136; R-109 to R-136; D-110 to R-136; L-111 to R-136; V-112 to R-136; F-113 to R-136; L-114 to R-136; L-115 to R-136; D-116 to R-136; D-117 to R-136; C-118 to R-136; N-119 to R-136; A-120 to R-136; L-121 to R-136; E-122 to R-136; Y-123 to R-136; P-124 to R-136; I-125 to R-136; P-126 to R-136; V-127 to R-136; T-128 to R-136; T-129 to R-136; V-130 to R-136; and L-131 to R-136 of SEQ ID NO:310. Polypeptides encoded by these polynucleotides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement there of are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to induce hematopoiesis), other functional activities (e.g., biological activities, ability to multimerize, ability to bind receptors, ability to activate receptors, ability to bind and block receptor activation, ability to

inhibit receptor activation without binding (e.g., as a dominant negative inhibitor of oligomeric complexes), ability to generate antibodies, ability to bind antibodies) may still be retained. For example the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxyl terminus of the amino acid sequence of the polypeptide shown in Figures 5A-5B (SEQ ID NO:310), as described by the general formula 1-n, where n is an integer from 6 to 135, where n corresponds to the position of the amino acid residue identified in SEQ ID NO:310. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: M-1 to Q-135; M-1 to R-134; M-1 to D-133; M-1 to P-132; M-1 to L-131; M-1 to V-130; M-1 to T-129; M-1 to T-128; M-1 to V-127; M-1 to P-126; M-1 to I-125; M-1 to P-124; M-1 to Y-123; M-1 to E-122; M-1 to L-121; M-1 to A-120; M-1 to N-119; M-1 to C-118; M-1 to D-117; M-1 to D-116; M-1 to L-115; M-1 to L-114; M-1 to F-113; M-1 to V-112; M-1 to L-111; M-1 to D-110; M-1 to R-109; M-1 to R-108; M-1 to C-107; M-1 to F-106; M-1 to S-105; M-1 to N-104; M-1 to M-103; M-1 to I-102; M-1 to T-101; M-1 to Y-100; M-1 to L-99; M-1 to K-98; M-1 to R-97; M-1 to A-96; M-1 to K-95; M-1 to D-94; M-1 to K-93; M-1 to L-92; M-1 to S-91; M-1 to D-90; M-1 to V-89; M-1 to Q-88; M-1 to A-87; M-1 to V-86; M-1 to K-85; M-1 to W-84; M-1 to C-83; M-1 to P-82; M-1 to P-81; M-1 to S-80; M-1 to A-79; M-1 to V-78; M-1 to F-77; M-1 to D-76; M-1 to R-75; M-1 to L-74; M-1 to K-73; M-1 to D-72; M-1 to L-71; M-1 to V-70; M-1 to C-69; M-1 to Y-68; M-1 to N-67; M-1 to H-66; M-1 to I-65; M-1 to D-64; M-1 to L-63; M-1 to Y-62; M-1 to L-61; M-1 to R-60; M-1 to P-59; M-1 to L-58;

M-1 to Y-57; M-1 to R-56; M-1 to V-55; M-1 to C-54; M-1 to P-53; M-1 to E-52; M-1 to S-51; M-1 to P-50; M-1 to E-49; M-1 to S-48; M-1 to V-47; M-1 to Q-46; M-1 to L-45; M-1 to L-44; M-1 to N-43; M-1 to F-42; M-1 to D-41; M-1 to R-40; M-1 to T-39; M-1 to I-38; M-1 to E-37; M-1 to Q-36; M-1 to S-35; M-1 to L-34; M-1 to A-33; M-1 to R-32; M-1 to M-31; M-1 to R-30; M-1 to S-29; M-1 to Y-28; M-1 to C-27; M-1 to T-26; M-1 to P-25; M-1 to P-24; M-1 to T-23; M-1 to P-22; M-1 to R-21; M-1 to A-20; M-1 to A-19; M-1 to P-18; M-1 to A-17; M-1 to G-16; M-1 to A-15; M-1 to L-14; M-1 to L-13; M-1 to L-12; M-1 to L-11; M-1 to L-10; M-1 to V-9; M-1 to P-8; and M-1 to L-7 of SEQ ID NO:310. Polypeptides encoded by these polynucleotides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:310, where n and m are integers as described above. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: M-1 to A-15; R-2 to G-16; T-3 to A-17; P-4 to P-18; G-5 to A-19; P-6 to A-20; L-7 to R-21; P-8 to P-22; V-9 to T-23; L-10 to P-24; L-11 to P-25; L-12 to T-26; L-13 to C-27; L-14 to Y-28; A-15 to S-29; G-16 to R-30; A-17 to M-31; P-18 to R-32; A-19 to A-33; A-20 to L-34; R-21 to S-35; P-22 to Q-36; T-23 to E-37; P-24 to I-38; P-25 to T-39; T-26 to R-40; C-27 to D-41; Y-28 to F-42; S-29 to N-43; R-30 to L-44; M-31 to L-45; R-32 to Q-46; A-33 to V-47; L-34 to S-48; S-35 to E-49; Q-36 to P-50; E-37 to S-51; I-38 to E-52; T-39 to P-53; R-40 to C-54; D-41 to V-55; F-42 to R-56; N-43 to Y-57; L-44 to L-58; L-45 to P-59; Q-

46 to R-60; V-47 to L-61; S-48 to Y-62; E-49 to L-63; P-50 to D-64; S-51 to I-65;  
 E-52 to H-66; P-53 to N-67; C-54 to Y-68; V-55 to C-69; R-56 to V-70; Y-57 to L-  
 71; L-58 to D-72; P-59 to K-73; R-60 to L-74; L-61 to R-75; Y-62 to D-76; L-63 to  
 F-77; D-64 to V-78; I-65 to A-79; H-66 to S-80; N-67 to P-81; Y-68 to P-82; C-69  
 5 to C-83; V-70 to W-84; L-71 to K-85; D-72 to V-86; K-73 to A-87; L-74 to Q-88;  
 R-75 to V-89; D-76 to D-90; F-77 to S-91; V-78 to L-92; A-79 to K-93; S-80 to D-  
 94; P-81 to K-95; P-82 to A-96; C-83 to R-97; W-84 to K-98; K-85 to L-99; V-86 to  
 Y-100; A-87 to T-101; Q-88 to I-102; V-89 to M-103; D-90 to N-104; S-91 to S-  
 105; L-92 to F-106; K-93 to C-107; D-94 to R-108; K-95 to R-109; A-96 to D-110;  
 10 R-97 to L-111; K-98 to V-112; L-99 to F-113; Y-100 to L-114; T-101 to L-115; I-  
 102 to D-116; M-103 to D-117; N-104 to C-118; S-105 to N-119; F-106 to A-120;  
 C-107 to L-121; R-108 to E-122; R-109 to Y-123; D-110 to P-124; L-111 to I-125;  
 V-112 to P-126; F-113 to V-127; L-114 to T-128; L-115 to T-129; D-116 to V-130;  
 D-117 to L-131; C-118 to P-132; N-119 to D-133; A-120 to R-134; L-121 to Q-135;  
 15 and E-122 to R-136 of SEQ ID NO:310. Polynucleotides encoding these  
 polypeptides are also encompassed by the invention. Moreover, fragments and  
 variants of these polypeptides (such as, for example, fragments as described herein,  
 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 these polypeptides and polypeptides encoded by the polynucleotide which  
 20 hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides, or the complement thereof are encompassed by the invention.  
 Antibodies that bind polypeptides of the invention are also encompassed by the  
 invention. Polynucleotides encoding these polypeptides are also encompassed by  
 the invention.

25 The present invention is also directed to proteins containing polypeptides at  
 least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a  
 polypeptide sequence set forth herein as m-n. In preferred embodiments, the  
 application is directed to proteins containing polypeptides at least 80%, 85%, 90%,  
 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid  
 30 sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides  
 encoding these polypeptides are also encompassed by the invention.

Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 97975 (deposited April 4, 1997) and ATCC Deposit No. 209081 (deposited May 29, 1997), where this portion excludes any integer of amino acid residues from 1 to about 606 (end of protein minus six) amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit No. 97975 and 209081, or any integer of amino acid residues from 6 to about 612 amino acids from the carboxyl terminus, or any combination of the above amino terminal and carboxyl terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97975 and 209081. Polypeptides encoded by these polynucleotides also are encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 4. Accordingly, polynucleotides related to this invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis for chromosome 4.

This gene is expressed primarily in fetal liver and fetal spleen.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic, immunological, developmental, and/or hepatic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and hematopoietic systems, expression of this gene at significantly higher or lower levels

may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune, hepatic, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. For example, polynucleotides and polypeptides of the invention, polynucleotide and polypeptide fragments, and polynucleotide and polypeptide variants, and antibodies directed to these polypeptides are useful for identifying, selecting, targeting and/or stimulating proliferation of hematopoietic stem cells (a.k.a., hematopoietic progenitor cells).

Cytokines typically exert their respective biochemical and physiological effects by binding to specific receptor molecules. Receptor binding then stimulates specific signal transduction pathways (Kishimoto, T., *et al.*, *Cell* **76**:253-262 (1994)).

The specific interactions of cytokines with their receptors are often the primary regulators of a wide variety of cellular processes including activation, proliferation, and differentiation (Arai, K. -I, *et al.*, *Ann. Rev. Biochem.* **59**:783-836 (1990); Paul, W. E. and Seder, R. A., *Cell* **76**:241-251 (1994)).

The polynucleotides and polypeptides of this invention may be useful for the diagnosis and treatment of a variety of immune system and hematopoietic disorders, pathologies, and/or deficiencies. For example, this gene and/or gene product may play a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Furthermore, polypeptides of this invention may be involved in the regulation of cytokine production, antigen presentation, or other processes useful for treatment of cancer, particularly leukemia (e.g., by boosting immune responses, suppressing hyperproliferative activity, or enhancing recovery of healthy hematopoietic cell populations during or following chemotherapy). Moreover, the polynucleotides and polypeptides of this invention, as well as antibodies against the polypeptides of this invention, may be useful for treating immunological and hematopoietic disorders; such as for examples, arthritis, asthma, immunodeficiency diseases (e.g. AIDS), leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia,

neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the polypeptide of this invention represents a secreted factor that is likely to have activity in stimulating the differentiation of blood cells, or recruiting immune and hematopoietic cells to sites of injury. Thus, this polypeptide is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more of the immunogenic epitopes shown in SEQ ID NO: 310 as residues: Met-1 to Leu-7, Pro-18 to Cys-27, Ser-29 to Ser-35, Glu-37 to Asp-41, Gln-46 to Cys-54, Asp-72 to Val-78, Pro-81 to Trp-84, Ser-91 to Lys-98, Asn-104 to Leu-111, Asp-116 to Leu-121, and Val-130 to Arg-136. Polynucleotides encoding said polypeptides are also encompassed by the invention. Antibodies that bind said epitopes or other polypeptides of the invention are also encompassed.

The tissue distribution of this gene in fetal liver and spleen indicates that the gene could be important for the treatment or detection of immune or hematopoietic disorders including arthritis, leukemia, and immunodeficiency diseases. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Moreover, expression within fetal tissue indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and



treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:72 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 982 of SEQ ID NO:72, b is an integer of 15 to 996, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 63**

This gene shares homology with human serum amyloid protein (See Genbank Accession No. W13671).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

ALTRIPPGDWVINVTAVSFAGKTTARFFHSSPPSLGDQARTDPGHQRRD (SEQ ID NO:711), SMLLLFPLQERPQQDSFIRLLLAWGTRLELTLDIKGGI (SEQ ID NO:712),

TGLWADGFSSHIIPPLMSRVSSSLVPQARRRRMKESCCGLSCKGNSSNIDYPV TGRNSCERAPLCAFALHFQERTXITGXGEDPGPFQSGXGRVTASRXTLACSHV

AMTPAGCXQALGTPSSYCVRKAPRA (SEQ ID NO:713), and/or QARRRRMKESCCGLSCKGNSSNIDYPVT (SEQ ID NO:714). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as

described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 9. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 9.

This gene is expressed primarily in fetal liver and spleen.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic, immune, and/or developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene in fetal liver-spleen indicates that the gene is important for the treatment or detection of immune or hematopoietic disorders including arthritis, leukemia, and immunodeficiency diseases. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene

product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency, etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, expression within fetal tissue indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:73 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 771 of SEQ ID NO:73, b is an integer of 15 to 785, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:73, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 64**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: LWRSSGVER (SEQ ID NO:715). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide

encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome

- 5 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed specifically in the brain.

- Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
 10 and for diagnosis of diseases and conditions which include, but are not limited to, neural disorders, particularly neurodegenerative disease states. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the  
 15 neurological systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression  
 20 level in healthy tissue or bodily fluid from an individual not having the disorder.

- The tissue distribution in brain indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis,  
 25 demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In  
 30 addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal

differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:74 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1055 of SEQ ID NO:74, b is an integer of 15 to 1069, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 65**

This gene shares homology with a yeast protein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

LQEVNITLPENSVWYERYKFDIPVFHL (SEQ ID NO:716). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention. (See Genbank Accession No. 1332638).

This gene is expressed primarily in fetal tissue (fetus and fetal liver).

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatic, developmental, immune, and/or hematopoietic disorders, including cancers (e.g. hepatoblastoma). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hepatic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, developmental, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 313 as residues: Asn-72 to Glu-77.

The tissue distribution in fetal liver indicates that the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed

progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:75 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 817 of SEQ ID NO:75, b is an integer of 15 to 831, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:75, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 66**

This gene has homology with a B-cell surface antigen which may indicate that this gene plays a role in the immune response, including, but not limited to disorders and infections of the immune system.

Preferred polynucleotide fragments comprise the following sequence:  
GTAGCATGTAGCCAGTCGAATAACNTATAAGGACAAAGTGGAGTCCACGC  
GTGCGCCGTCTAGACTAGTGGATCCCCCGGCTGCAGGATTCGGCACGAG  
(SEQ ID NO:718). Also preferred are polypeptides comprising polypeptide fragments encoded by these polynucleotide fragments.

This gene shares homology with an interferon-gamma receptor (See Genbank Accession No.T94535).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
MQGSGSQFRACLLCLCFSCPCSPGGPRWNSRQGGRFPKTCRAISQNLVFKY  
KTFCPVRYMQPHRSSLLHFTSYVFILSTWGLRTYSTDLKKKKKNSRGGPVP

IRPKS (SEQ ID NO:717),

MQGSGSQFRACLLCLCFSCPCSPGGPRWNSRQGGRFPKTCRAISQNLVFK  
(SEQ ID NO:719),

PVRYMQPHRSSLCLHFTSYVFILSTWGSLRTYSTDLKKKKKNSRGGPVPIRPK

5 S (SEQ ID NO:720),

GEEQRDCSLGWRGVGMRATHCQAARMFVLFSLPKYAGL (SEQ ID NO:721),

TSGSPGCRIRHELPGEEQRDCSLGWRGVGMRATHCQAAR (SEQ ID NO:722),

EPPIAKQQECSCFFPFQNMQGSGSQFRACLLCLCFSCPCSPGGPRWNSRQGR

RFPKTCRAISQNLVFKYKTFCPVRYMQPHRSSLCLHFTSYVFILSTWGSLRTY

10 STDLKKKKKNSRGGPVPIRPKS (SEQ ID NO:723), and/or

QFRACLLCLCFSCPCSPGGPRWNSRQGGRF (SEQ ID NO:724). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide

15 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

This gene is expressed primarily in T-cells and gall bladder.

20 Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
immunological disorders and conditions (immunodeficiencies, cancer, leukemia,  
hematopoiesis), in addition to metabolic, gastrointestinal, and/or digestive disorders.

25 Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
providing immunological probes for differential identification of the tissue(s) or cell  
type(s). For a number of disorders of the above tissues or cells, particularly of the  
immune and digestive systems, expression of this gene at significantly higher or  
lower levels may be routinely detected in certain tissues or cell types (e.g., immune,  
30 hematopoietic, metabolic, gastrointestinal, digestive, and cancerous and wounded  
tissues) or bodily fluids (e.g., lymph, serum, bile, plasma, urine, synovial fluid and  
spinal fluid) or another tissue or cell sample taken from an individual having such a



disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 314 as residues: Thr-41 to Gly-52.

The tissue distribution in T-cells indicates that the protein product of this gene is useful for the treatment and diagnosis of immune disorders including: leukemias, lymphomas, auto-immune disorders, immunosuppressive (transplantation) and immunodeficiencies (e.g. AIDS), inflammation and hematopoietic disorders.

Moreover, the expression of this gene in gall bladder would suggest a possible role for this gene product in digestive disorders, particularly of the pancreas or liver.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:76 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 576 of SEQ ID NO:76, b is an integer of 15 to 590, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 67**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

NQFTSCILFCDGGHWRELLFQSI (SEQ ID NO:725),  
 AMSSKLLNLLALLQYSVHDHCHPRLLKRGARATLRHKGWGPSSLRGCESF  
 QIVLIGWGPDLAVGFGRGKLLSRSLPVRHGGVSEFCLPHRDVVRLEKVKK  
 (SEQ ID NO:726), and/or GPSSLRGCESFQIVLIGWGPDLAVGFGRGKLLS (SEQ

ID NO:727). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed primarily in a variety of fetal and developmental tissues (e.g. fetal spleen, infant brain).

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental, immune or neurological abnormalities. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developing immune and central nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, immune, hematopoietic, hepatic, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 315 as residues: Ser-38 to Ser-43.

The tissue distribution in fetal tissues indicates that the protein product of this gene is useful for developmental abnormalities or fetal deficiencies. The detection in infant brain would suggest a role in neurological disorders (both developmental and neurodegenerative conditions of the brain and nervous system, behavioral disorders,

depression, schizophrenia, Alzheimer's disease, Parkinson's disease, Huntington's disease, mania, dementia). In addition, the detection in spleen would similarly suggest a role in the detection and treatment of immune disorders (e.g. immunodeficiency, inflammation, cancer, wound healing, tissue repair,

- 5 hematopoiesis). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:77 and may have been publicly available prior to conception of  
 10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1260 of SEQ ID NO:77, b is an  
 15 integer of 15 to 1274, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:77, and where b is greater than or equal to a + 14.

#### *FEATURES OF PROTEIN ENCODED BY GENE NO: 68*

20

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

TRKNIDFXETEKYYLFSFSNNVSFKNFWLKYN (SEQ ID NO:728). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as  
 25 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this  
 30 polypeptide are also encompassed by the invention.

This gene is expressed primarily in spleen, T-cells, and fetal heart.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunological or hematopoietic deficiencies or disorders, including AIDS and cardiovascular or developmental conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and cardiovascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, cardiovascular, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in spleen and T-cells indicates that the protein product of this gene is useful for the diagnosis and treatment of immune disorders including: leukemias, lymphomas, autoimmune disorders, immunodeficiencies (e.g. AIDS), immunosuppressive conditions (transplantation) and hematopoietic disorders.

Moreover, the expression in fetal heart indicates that the protein product of this gene is useful for the treatment and diagnosis of cardiovascular disorders (e.g. heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:78 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1119 of SEQ ID NO:78, b is an

integer of 15 to 1133, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:78, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 69

This gene shares homology with a human collagen protein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 10 MPRKTSKCRQLLCSGASRNADTAARQSTCSSHRPPGKIPSLGPRRXPGCXSVPS  
SSRGEQSTGSPAAPRCGRRDAHRGLPGGAAMTPGDTWASFNPRAGHSKSQGE  
EGQESSGASRQDRHPVSHWVERQREAWGAPRSSSAGGVKVAATTEREPEFKI  
KTGKA (SEQ ID NO:729),  
CSGASRNADTAARQSTCSSHRPPGKIPSLGPRRXPGCXSVPSRGEQSTGSPA
- 15 APRCGRRDAHRGLPGGAAMTPGDTWASFNPRAGHS (SEQ ID NO:730),  
QGEGQESSGASRQDRHPVSHWVERQREAWGAPRSSSAGGVKVAATTEREPE  
FKIKTGKA (SEQ ID NO:731),  
IRHEGKRMLNESRKPLSFASRLSSLYFKLGFPCGRSNLYSTCTAAPGGSPGLP  
LPFYPVADG (SEQ ID NO:732),
- 20 TRAESLFPLLHAFPVFILNSGSLSVVAATFTPPALLLLGAPQASLCLSTQWLTG  
CLSCLDAPLLSCPSPWLLLCALGLKLAHVSPGVMAAPPGRPLCASRLPHLGA  
AGEPVLCSPRLLGTQLPGXLRGPRGILPGGRWEEQVLCLAAVSAFLDAPEH  
RSCRHFEVFLGMCQIT (SEQ ID NO:733),  
PALGLKLAHVSPGVMAAPPGRPLCASRLP (SEQ ID NO:734),
- 25 GGRWEEQVLCLAAVSAFLDAPEHR (SEQ ID NO:735),  
SWPMCPPEWLLLLGGLCVRHVFHTWGQLASPCSVPLGCLAQSCSLGXSVDP  
DWGFCQGGDGRSRCFAWRLCLHFWTPQSTEVAGTLRSSSACARLHE (SEQ  
ID NO:736), and/or GDGRSRCFAWRLCLHFWTPQSTEVAGTLR (SEQ ID  
NO:737). Moreover, fragments and variants of these polypeptides (such as, for
- 30 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal heart.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cardiovascular or developmental disorders, particularly vascular conditions.

10 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cardiovascular, developmental, skeletal, vascular, and cancerous and wounded tissues) or bodily

15 fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID

20 NO: 317 as residues: Pro-32 to Ser-39.

The tissue distribution in fetal heart indicates that the protein product of this gene is useful for the treatment and diagnosis of cardiovascular disorders (e.g. heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis), in addition to vascular disorders, such as microvascular disease. Expression within fetal tissue

25 indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer

30 therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

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Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:79 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 647 of SEQ ID NO:79, b is an integer of 15 to 661, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 70

The translation product of this gene shares sequence homology with a chicken single-strand DNA-binding protein. The promoter region of the chicken alpha2(I) collagen gene contains a pyrimidine-rich element that is well conserved in different mammalian species. This sequence can also form an unusual DNA structure as shown by its sensitivity to SI nuclease in vitro and it lies in a region that is DNase I-hypersensitive only when this promoter is active. The high affinity of this protein for this conserved pyrimidine-rich region indicates that it might be involved in the transcriptional regulation of the alpha2(I) collagen gene.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MSPRYPGGPRPPLRIPNQALGGVPGSQPLLPSGMDPTRQQGHPNMGGPMQR  
MTPPRGMVPLGPQNYGGAMRPPLNALGGPGMPGMNMGPGGGRPWPNTN  
ANSIPYSSASPGNYVGPPGGGGPPGTPIMPSPADSTNSGDNMYTLMNAVPPGP  
NRPNFPMGPGSDGPMGGLGGMESHMNGSLGSGDMDSISKNSPNNMSLSNQ  
PGTPRDDGEMGGNFLNPFQSESYSPSMTMSV (SEQ ID NO:738),  
MSPRYPGGPRPPLRIPNQALGGVPGSQPLLPSGMDPTRQQGHPNMGGPMQR  
MTPPRGMVPLGPQNYGGAMRPPLNALGGPGMPGMNMGPGGGRPWPNTN  
ANSIPYSSASPGNY (SEQ ID NO:739),

LNALGGPGMPGMNMGPGGGRPWPNPNTNANSIPYSSASPGNYVGPPGGGGPP  
 GTPIMPSPADSTNSGDNMYTLMNAVPPGPN (SEQ ID NO:740),  
 GPMGGLGGMESHMNGSLGSGDMDISISKNPNNMSLSNQPGTPRDDGEMG  
 GNFLNPFQSESYSPSMTMSV (SEQ ID NO:741), TCEHSSEAKAFHDY (SEQ ID  
 5 NO:742), and/or RRETCEHSSEAKAFHDYPF (SEQ ID NO:743),. Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
 10 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention. (See Genbank Accession  
 No. 1562534)

This gene is expressed primarily in placenta, and to a lesser extent, in fetal  
 15 heart.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 developmental abnormalities, fetal deficiencies, and particularly of the cardiovascular  
 20 system and/or vascular conditions. Similarly, polypeptides and antibodies directed to  
 these polypeptides are useful in providing immunological probes for differential  
 identification of the tissue(s) or cell type(s). For a number of disorders of the above  
 tissues or cells, particularly of the reproductive system, expression of this gene at  
 significantly higher or lower levels may be routinely detected in certain tissues or cell  
 25 types (e.g., developmental, vascular, cardiovascular, reproductive, and cancerous and  
 wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine,  
 synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
 individual having such a disorder, relative to the standard gene expression level, i.e.,  
 the expression level in healthy tissue or bodily fluid from an individual not having the  
 30 disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID  
 NO: 318 as residues: Met-1 to Leu-13, Gly-33 to Gly-46, Pro-48 to Gly-57, Pro-63 to

"032201" 2926669



Gly-68, Pro-89 to Asn-102, Ser-108 to Asn-113, Pro-118 to Pro-124, Pro-132 to Asn-141, Pro-151 to Asn-157, Ile-191 to Met-199, Ser-202 to Gly-215, Phe-222 to Pro-229.

The tissue distribution in fetal heart and placenta indicates that the protein product of this gene is useful for the detection and treatment of developmental abnormalities or fetal deficiencies, ovarian and other endometrial cancers, reproductive dysfunction, cardiovascular disorders, and pre-natal disorders, in particular vascular disorders, which include, but are not limited to, stroke, angina, microvascular disease, atherosclerosis, embolism, and aneurysm. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:80 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1364 of SEQ ID NO:80, b is an integer of 15 to 1378, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 71**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: TITLFQSAWCFFSKYCTDFT (SEQ ID NO:744), VRGCEDGGGGGIWGGWWPGQQMAPPWLSCPHRQFPHFHSGRQRRQSDLLK EELPQPSGAAGRASGNKPYTPPPASNSLTLRLLSFRFNAFNRSHPQPSLNKYD RQ (SEQ ID NO:745), PWLSCPHRQFPHFHSGRQRRQSDLL (SEQ ID NO:746), and/or RLLSFRFNAFNRSHPQPSLN (SEQ ID NO:747). Moreover, fragments and

variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in fetal liver, and to a lesser extent, in the breast and testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatic disorders (including hepatoblastomas), hematopoietic, immune, and/or reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hepatic and reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic, immune, hepatic, reproductive, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal liver indicates that the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). The expression in testes and breast indicates that the protein product of this gene is useful for the

detection and treatment of endocrine and reproductive disorders (e.g. sperm maturation, milk production, testicular and breast cancers). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:81 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1426 of SEQ ID NO:81, b is an integer of 15 to 1440, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where b is greater than or equal to a + 14.

## 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 72**

In specific embodiments, polypeptides of the invention comprise, or  
20 alternatively consists of, an amino acid sequence selected from the group:  
RDSSLWAAALSFRQQCSSLASCLVSMYSRPGRQHRAKAGAGSQTEQCWGRK  
VDAVV (SEQ ID NO:748), CLVSMYSRPGRQHRAKAGAGSQTEQCW (SEQ ID  
NO:749),  
PEHGFSSCDFWEGAPSSGPKEGGRSPPQLACVWGMNLSSPPCLALLTNRACL  
25 AVNWHRVTLFPGIQVCNQNTGEEKLQDPCPHLSS (SEQ ID NO:750),  
RSPPQLACVWGMNLSSPPCLALLTNRACLA (SEQ ID NO:751),  
CERDSETSSIAMTCIKHKPPKQKKRLSLLPGFRSALPRVCRCHMITVQREAFRT  
HTGCSTSVHLPSRGGFLPDF (SEQ ID NO:752), and/or  
KKRLSLLPGFRSALPRVCRCHMITVQRE (SEQ ID NO:753).

30 Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the

polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 5           The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in smooth muscle, and to a lesser extent, in brain.

- 10           Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cardiovascular and neurological disorders, particularly embolism, atherosclerosis, stroke, aneurysm, and microvascular disease. Similarly, polypeptides and antibodies
- 15   directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular and central nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, vascular, endothelial,
- 20   smooth muscle, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

- 25           The tissue distribution in brain and smooth muscle indicates that the protein product of this gene is useful for the detection and treatment of restenosis, atherosclerosis, stroke, angina, thrombosis, wound healing and other conditions of heart disease. Moreover, the protein product of this gene is useful for the detection and treatment of developmental, degenerative and behavioral conditions of the brain
- 30   and nervous system (e.g. schizophrenia, depression, Alzheimer's disease, Parkinson's disease, Huntington's disease, mania, dementia, paranoia, addictive behavior and

sleep disorders). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:82 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1367 of SEQ ID NO:82, b is an integer of 15 to 1381, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:82, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 73**

This gene shares homology with human stromalin-2, which is believed to play an integral role in modulating cellular function of hematopoietic cells and tissues, and may possibly serve as a tumor suppressor.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

QAFVLLSDLLLIFSPQMIVGGRDFLRPLVFFPEATLQSELASFLMDHVFIQPGD  
 LGSGA (SEQ ID NO:754),  
 ACSYLLCNPEFTFFSRADFARSQVLDLLTDRFQQELEELLQVG (SEQ ID  
 NO:755), QKQLSSLRDRMVAFCCLCQSCLSDVDTEIQEQVST (SEQ ID  
 NO:756), QVILPALTLVYFSILWTLTHISKSDAS (SEQ ID NO:757),  
 STHDLTRWELYEPCCQLLQKAVDTGXVPHQV (SEQ ID NO:758),  
 TSFLFPLQAFVLLSDLLLIFSPQMIVGGRDFLRPLVFFPEATLQSELASFLMDH  
 VFIQ PGDLGSGA (SEQ ID NO:759),  
 GWGACSYLLCNPEFTFFSRADFARSQVLDLLTDRFQQELEELLQVGAGAGQ  
 WDTPNKGGRGCKTGDVD (SEQ ID NO:760),  
 VWVLDGIMGTEESVSSFFPKPLCPQKQLSSLRDRMVAFCCLCQSCLSDVDTE

IQEQVSTDSSGSNKASIPAPIPRRN (SEQ ID NO:761),  
 NASLPSTSEWLSSSSPSRFYWCLWSWFPLFFSSITFPFLPQSTHDLTRWELYEP  
 CCQLLQKAVDTGXVPHQVSGQARDGLGAGGLXFKDLRSRWPLGVSSLSAW  
 SGQSEEDQVGGGHLLHSSLRRWTLLPGSSWISWKPRIILRDSRRRRVN (SEQ  
 5 ID NO:762), VLGEMLLWIFFPSQSSFLDEDEVYNLAATLKRLSAFYK (SEQ ID  
 NO:763), PKPHFSNPLLLQVILPALTLVYFSILWTLTHISKSDASPGECGS (SEQ  
 ID NO:764), and/or HCQFLLG (SEQ ID NO:765). Moreover, fragments and  
 variants of these polypeptides (such as, for example, fragments as described herein,  
 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 10 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
 under stringent conditions, to the polynucleotide encoding these polypeptides ) are  
 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 also encompassed by the invention. (See Genbank Accession No.R65208 )

15 The gene encoding the disclosed cDNA is believed to reside on chromosome  
 7. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 7.

This gene is expressed primarily in the brain (infant brain, adult brain,  
 pituitary, cerebellum, hippocampus, schizophrenic hypothalamus, amygdala).

20 Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 developmental disorders and neurodegenerative diseases of the brain and nervous  
 system, in addition to immune or hematopoietic disorders. Similarly, polypeptides  
 25 and antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of  
 disorders of the above tissues or cells, particularly of the central nervous system,  
 expression of this gene at significantly higher or lower levels may be routinely  
 detected in certain tissues or cell types (e.g., neural, developmental, immune,  
 30 hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
 amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue  
 or cell sample taken from an individual having such a disorder, relative to the

standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 321 as residues: Thr-25 to Lys-36, Lys-55 to Ser-63.

5           The tissue distribution primarily in brain, combined with the homology to the highly conserved SA-1 and SA-2 proteins, indicates that the protein product of this gene is useful for the detection and treatment of developmental, degenerative and behavioral conditions of the brain and nervous system (e.g. schizophrenia, depression, Alzheimer's disease, Parkinson's disease, Huntington's disease, mania, dementia, paranoia, addictive behavior and sleep disorders). Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:83 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1692 of SEQ ID NO:83, b is an integer of 15 to 1706, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:83, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 74

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
EFGTSLVALELHELLYHWETRAQPSLILYVSDLRWMEFRTSCLLFDFVLFLE  
(SEQ ID NO:766),

TKPGMVGHVPIVPATKXAEAGGSPEPGSSTLQWPMITPCTPSWATEPDHVSE  
10 DE (SEQ ID NO:767), and/or LLYHWETRAQPSLILYVSDLRWMEFRTSC (SEQ  
ID NO:768).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
15 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the hypothalamus of a human suffering  
20 from schizophrenia.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the CNS, particularly schizophrenia. Similarly, polypeptides and  
25 antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, such as schizophrenia expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, and cancerous and wounded  
30 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a



disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 322 as residues: Gly-38 to Ala-44.

5 The tissue distribution in the hypothalamus indicates that the protein products of this gene are useful for the study, diagnosis and treatment of schizophrenia and other disorders involving the CNS. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's  
10 Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors,  
15 including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role  
20 in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:84 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
30 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 559 of SEQ ID NO:84, b is an

integer of 15 to 573, where both a and b correspond to the positions of nucleotide residues shown in SEO ID NO:84, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 75

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

LAVSTSFICCADISTALPLGSSRPAPAPRHREHEHGHQARPPRLXTSLMPLST

10 PAA AQLLWTQLTPMGG R PGGRHSPPTLHTGPRALPPGPPHPSLHVAALSLLR  
(SEQ ID NO:769).

APAVPHQPPGTESTSMGTPGLPGCSXRPLCHYQHQLXPSYFGHSSPPWG

AVLVGVTPHPRCTPAPGPCRLGLHHPCTWQLCLC (SEQ ID NO:770),

CADISTALPLGSSRPAPAPRHREHEHGH (SEQ ID NO:771),

15 WTQLTPMGGRPGGRHSPPTLHTGPR (SEQ ID NO:772), and/or HQPPGTEST  
SMGTKPGLPGC (SEQ ID NO:773). Moreover, fragments and variants of these

polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent

20 conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in endometrial tumors, and to a lesser extent,  
25 in amniotic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, developmental, and immune disorders, particularly cancers of those systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,

particularly of the reproductive and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 323 as residues: Ser-3 to Arg-9.

The tissue distribution in endometrium and amniotic cells indicates that the protein products of this gene are useful for the study and treatment of developmental, reproductive, and immune disorders, particularly cancers of those systems. Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:85 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 670 of SEQ ID NO:85, b is an integer of 15 to 684, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:85, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 76

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 SRGSLLPPHLPHRVVVRVHRGAKSLKALRQYIGAAHLQLPWDGKDPARPLGI  
 TLCLQMEIQVLG (SEQ ID NO:774),  
 CCSFGFYVMVGSDTAEKQGPIPGSQTQEGPWLSRHTHSPRAVPESSTAPAQ  
 PLLLPAPQARRWASNANGWGWDHQRREGQANYPYSPARPAPHNLHPQYLN  
 LHLQTQCYAQGSGWVLPPIG QLKVGGPYILPEGLQGLCSSVHPHNNPVR  
 (SEQ ID NO:775), HRGAKSLKALRQYIGAAHLQLPWDG (SEQ ID NO:776),  
 PAPQARRWASNANGWGWDHQR (SEQ ID NO:777), and/or  
 HPQYLNHLQTQCYAQGSGWVLP (SEQ ID NO:778).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 22. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 22.

This gene is expressed primarily in kidney cortex, and to a lesser extent, in early stage human brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, renal disorders such as renal cancer, developmental, or neural disorders, particularly cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,

particularly of the kidney expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, neural, renal, urogenital, endothelial, vascular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or  
 5 another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 324 as residues: Gly-38 to Gly-45, Gly-47 to Gly-52, Pro-92 to Lys-110.

10 The tissue distribution in kidney cortex indicates that the protein products of this gene are useful for the study, treatment and diagnosis of renal diseases, including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms  
 15 Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Moreover, the expression within human brain indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease,  
 20 Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in  
 25 feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment  
 30 and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Furthermore, the protein product may also show utility in the treatment and/or prevention of a variety

of vascular disorders, particularly embolism, aneurysm, stroke, atherosclerosis, or microvascular disease. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:86 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1022 of SEQ ID NO:86, b is an integer of 15 to 1036, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:86, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 77**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

TNGIMQYVTFVCVWLILFSIMFLRFIQAVACISTSFLFLAEYYSHWIYHNSFTYSS  
FVSAVWLL (SEQ ID NO:779), YNFMFNFSKNCQKVFHSGCIYIPTGNVQGFLF  
FHILALTNT SFXXXFCFFIATLVDVKWHLIVLICISLMTNDIILFLCAYGSK  
VFPWRNVPSSPLPFQNLVICLLLSF KKFVPGAV AHL (SEQ ID NO:780),  
CVTQARVQWRDLGSLQPPPPGFKRFSCLSLLSRXDYMHLPPRPANFCIFSKM  
GFHHVGQAGLEV LXSSDL PALASQSAXITGEPLRLARIS (SEQ ID NO:781),  
LILFSIMFLRFIQAVACISTSFLF (SEQ ID NO:783), and/or LPPRPANFCIFSK  
MGFHHVGQAGLE (SEQ ID NO:782). Moreover, fragments and variants of these  
polypeptides (such as, for example, fragments as described herein, polypeptides at  
least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
and polypeptides encoded by the polynucleotide which hybridizes, under stringent

conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

5           This gene is expressed primarily in kidney medulla.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, metabolic and renal disorders. Similarly, polypeptides and antibodies directed to  
10 these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the metabolic and renal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., renal, urogenital, endocrine, and cancerous and wounded  
15 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in kidney tissue indicates that the protein products of  
20 this gene are useful for study, treatment and diagnosis of metabolic and renal diseases and disorders. Moreover, this gene or gene product could be used in the treatment and/or detection renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms  
25 Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:87 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 894 of SEQ ID NO:87, b is an integer of 15 to 908, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:87, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 78

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

ALVPSPQQILPSCFSLMWQVTTKSALVFFKCIYIPFLSAPSLPRLNCLIFCSLD  
VQSQLVFLSSPPVAGVLFFLLSPLGSKSCSTVEX (SEQ ID NO:784), and/or

APSLPRLNCLIFCSLDVQSQLVFLS (SEQ ID NO:785). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in chronic synovitis and microvascular endothelium.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, skeletal or vascular disorders, such as arthritis and atherosclerosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular and skeletal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skeletal, synovium,



endothelial cells, vascular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in synovium and microvascular endothelium indicates that the protein products of this gene are useful for study, diagnosis and treatment of arthritic and other inflammatory diseases as well as cardiovascular diseases.

Moreover, the expression of this gene product in synovium would suggest a role in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis, bone cancer, as well as, disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). In addition, the protein would also be useful in the treatment and/or prevention of a variety of vascular disorders, which include, but are not limited to, microvascular disease, embolism, thrombosis, aneurysm, stroke, or atherosclerosis. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:88 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 641 of SEQ ID NO:88, b is an integer of 15 to 655, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:88, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 79

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: SSPSRVRLRHTPG (SEQ ID NO:786), and/or  
SNTNYCFMFFYFPVKVLVPFKNCYILSLILPCCICGHQFPRXQACTFCLHTLG  
10 GFSFSXLFLVLLSFYVQTGFSV (SEQ ID NO:787). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are  
15 also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in resting T-cells and activated monocytes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
20 and for diagnosis of diseases and conditions which include, but are not limited to, immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at  
25 significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily  
30 fluid from an individual not having the disorder.

The tissue distribution in T-cells and monocytes indicates that the protein products of this gene are useful for the study and treatment of immune diseases such

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as inflammatory conditions. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:89 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1088 of SEQ ID NO:89, b is an integer of 15 to 1102, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:89, and where b is greater than or equal to a + 14.

### 30 FEATURES OF PROTEIN ENCODED BY GENE NO: 80

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

GTSRHGQRPIAPGTPWQREPRVEVMDPAGGPRGVLP RPCRXLVLLNPRGGKG  
KALQLFRSHVQPLLAEAEISFTLMLTERRNHARELVRSEELGRWXALVVMXG

5 D GLMHEVVNGLHGAA (SEQ ID NO:788), and/or

RPIAPGTPWQREPRVEVMDPAGGP (SEQ ID NO:789). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

This gene is expressed in a variety of immune system tissues, e.g., neutrophils, T-cells, and TNF induced epithelial and endothelial cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, infectious and immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and vascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 328 as residues: Met-1 to Trp-6.

The tissue distribution in immune tissues and cells indicates that the protein products of this gene are useful for the study and treatment of infectious diseases, immune and vascular disorders. Moreover, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:90 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1519 of SEQ ID NO:90, b is an integer of 15 to 1533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:90, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 81

5           In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: ASGPLMGXAVLKIFE (SEQ ID NO:790). Polynucleotides encoding these polypeptides are also encompassed by the invention.

          This gene is expressed in activated neutrophils.

10           Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammation and other immune or hematopoietic conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological  
15 probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal  
20 fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

          The tissue distribution in neutrophils indicates that the protein products of this gene are useful for the study and treatment of immune disorders. Moreover, this gene  
25 product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including  
30 arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated

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cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:91 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 561 of SEQ ID NO:91, b is an integer of 15 to 575, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:91, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 82

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
 LLRSALXSPHLPTVPVLV (SEQ ID NO:791),  
 QXRNLAQEAFKWIPQDRPTVRSRMRGLSIRLPILASNCCALPFXXTSPLQC  
 LWSCHCSFQANTGLAS (SEQ ID NO:792),  
 QMTQEPTSVRAHGIAAWGNGCRDKNTKRLIQYWPESCSGMTKGTGVGRW  
 GEXRAERSS (SEQ ID NO:793), and/or HGIAAWGNGCRDKNTKRLIQY (SEQ  
 ID NO:794). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,

96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

- 5 Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in neutrophils.

- Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammatory and other immune or hematopoietic conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain
- 10 tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in
- 15 healthy tissue or bodily fluid from an individual not having the disorder.

- 20 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 330 as residues: Ala-83 to Thr-91.

- The tissue distribution in neutrophils indicates that the protein products of this gene are useful for the study and treatment of immune disorders. Moreover, the expression of this gene product in neutrophils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of hematopoietic cell
- 25 lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene
- 30 product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease,



inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:92 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 625 of SEQ ID NO:92, b is an integer of 15 to 639, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:92, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 83

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: CERSGYTRMAMDT (SEQ ID NO:795),  
TGSILAVGKKYSLGSYSRGDWHMRVVGLRGLGASTLQGLLIGIKPNKPQGRG  
KLQGRSSRKDTVLWPSPEHPHVMVMAILVYPDL SHYSNPHSTPAALLGCWPP  
FREGEILGLQRPGQWPEERC DRPWLPPC (SEQ ID NO:796),  
GSYSRGDWHMRVVGLRGLGASTLQGLLIG (SEQ ID NO:797), and/or

STPAALLGCWPPFREGEILGLQRPQW (SEQ ID NO:798). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in human neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammation and immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and inflammatory system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that the protein products of this gene are useful for diagnosis and treatment of disorders of the inflammatory and immune systems. Moreover, expression of this gene product in neutrophils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be

also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity;

5 immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells

10 and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:93 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

20 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 844 of SEQ ID NO:93, b is an integer of 15 to 858, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:93, and where b is greater than or equal to a + 14.

#### 25 **FEATURES OF PROTEIN ENCODED BY GENE NO: 84**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

30 TMGTWVDWLTTNTAHTPAIAAAICAEDFPQRHCGSVSPDQAC (SEQ ID NO:799), and/or TNTAHTPAIAAAICAEDFPQRHC (SEQ ID NO:800). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as

described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed in human neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammatory and immune or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the inflammatory and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that the protein products of this gene are useful for diagnosis and treatment of disorders of the immune and inflammatory systems. Moreover, the expression of this gene product indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis,

granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:94 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 512 of SEQ ID NO:94, b is an integer of 15 to 526, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:94, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 85**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: MSPETKGKGRSFPLK (SEQ ID NO:801),

CQNKCSSETTCGRTRRESNKQARAMAFIFKGKDLPPFFVSGDIQPKSSGSMAPD

QQGLCYLGSWRSHLYCRLLPMDQVSPALC (SEQ ID NO:802),

KPSPGLAYCSLSWSFHMLFLNICSGITIPVILSSGPSHLSTLSLAVSPRRPGTWV

KACSCWCP (SEQ ID NO:803), NKQARAMAFIFKGKDLPPFFVSGDI (SEQ ID

NO:804), YLGSWRSHLYCRLLPMDQVSP (SEQ ID NO:805), and/or  
 GITIPVILSSGPSHLSTLSLAVSPR (SEQ ID NO:806). Moreover, fragments and  
 variants of these polypeptides (such as, for example, fragments as described herein,  
 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 5 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
 under stringent conditions, to the polynucleotide encoding these polypeptides ) are  
 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 also encompassed by the invention.

10 This gene is expressed in activated neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 inflammation and immune or hematopoietic diseases. Similarly, polypeptides and  
 15 antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of  
 disorders of the above tissues or cells, particularly of the immune system and  
 inflammatory system, expression of this gene at significantly higher or lower levels  
 may be routinely detected in certain tissues or cell types (e.g., immune,  
 20 hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
 serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
 taken from an individual having such a disorder, relative to the standard gene  
 expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
 individual not having the disorder.

25 The tissue distribution in neutrophils indicates that the protein products of this  
 gene are useful for diagnosis and treatment of diseases of the inflammatory and  
 immune systems. Moreover, the expression of this gene product indicates a role in  
 the regulation of the proliferation; survival; differentiation; and/or activation of  
 hematopoietic cell lineages, including blood stem cells. This gene product may be  
 30 involved in the regulation of cytokine production, antigen presentation, or other  
 processes that may also suggest a usefulness in the treatment of cancer (e.g. by  
 boosting immune responses). Since the gene is expressed in cells of lymphoid origin,

the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:95 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 412 of SEQ ID NO:95, b is an integer of 15 to 426, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:95, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 86

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: LERLGVGRGLE (SEQ ID NO:807),  
DLPPCWTTLKEHQCFMQYQLFTIQCKVVEQTICEDERKMESTCLTLXPESV

RQXCPATLWSSMNIC (SEQ ID NO:808), and/or

TNRVXLSWRKEEQRMGRTETGAKDKGRDFLERGSRGWQLYTGAADTEEV

(SEQ ID NO:809) . Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,

5 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the  
10 invention.

This gene is expressed in activated neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
15 inflammation and immune system disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the inflammatory and immune system, expression of this gene at significantly higher or lower levels may be routinely  
20 detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 334 as residues: Met-1 to Gly-6, Gly-32 to Pro-43, Leu-55 to Gln-60.

The tissue distribution in neutrophils indicates that the protein products of this gene are useful for diagnosis and treatment of disorders of the immune and inflammatory system. Moreover, the expression of this gene product indicates a role  
30 in the regulation of the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other

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processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma,

5 immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility,

10 lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the

15 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:96 and may have been publicly available prior to conception of

20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 830 of SEQ ID NO:96, b is an

25 integer of 15 to 844, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:96, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 87**

30 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

EQVLALLWPRFELILEMNVQSVRSTDPQRLGGLDTRPHYITRRYAEFSSALVSI  
 NQTIPNERTMQLLGQLQVEVENFVLRVAAEFSSRKEQLVFLINNYDMMLGVL  
 MERAADDSKEVESFQQLLNARTQEFIEELLSPFGLVAFVKEAEALIERGQA  
 ERLRGEEARVTQLIRGFGSSWKSSVESLSQDVMRSFTNFRNGTSIIQG (SEQ ID

5 NO:810), ALLKYRFFYQFLGNERATAKEIRDEYVETLSKIYLSYYRSYL  
 GRMLMKVQYEEVAEKDDLGMGVEDTAKKGFXSKPSLRSRNTIFTLGTRGSVIS  
 TELEAPILVPHTAQR (SEQ ID NO:811),

EQRYPFEALFRSQHYXLLDNSCREYLFICEFFVVS GPXAHDLFHAVMGRTLS  
 MTLKHLD SYLADCYDAIAVFLCIHIVLRFRNIAAKRDVPALDRYW (SEQ ID  
 10 NO:812), GGLDTRPHYITRRYAEFSSALVSINQ (SEQ ID NO:813),

SRKEQLVFLINNYDMMLGVL (SEQ ID NO:814),

ALLKYRFFYQFLGNERATAKEIRDEYVETLSKIYLSYYRSYLGRMLMKVQYE  
 EVAEKDDLGMGVEDTAKKGFXSKPSLRSRNTIFTLGTRGSVISPTLEAPILVPH  
 TAQRXEQRYPFALFRSQHYXLLDNSCREYLFICEFFVVS GPXAHDLFHAVM  
 15 GRTLSMTLKHLD SYLADCYDAIAVFLCIHIVLRFRNIAAKRDVPALDRYWEQ  
 VLALLWPRFELILEMNVQSVRSTDPQRLGGLDTRPHYITRRYAEFSSALVSIN  
 QTIPNERTMQLLGQLQVEVENFVLRVAAEFSSRKEQLVFLINNYDMMLGVL  
 MERAADDSKEVESFQQLLNARTQEFIEELLSPFGLVAFVKEAEALIERGQA  
 ERLRGEEARVTQLIRGFGSSWKSSVESLSQDVMRSFTNFRNGTS (SEQ ID

20 NO:815),  
 PADLRAVSGTSEVGLMMLLELHHKVVNVDELSPGREGSELRLGQHPVEAMIEL  
 DQLGQRSLNDTGAISEVGETPHYILTQRFH (SEQ ID NO:816), and/or  
 GPHPGASHSAAXEQRYPFALFRSQHYXLLDNSCREYLFICEFFVVS GPXAH  
 LFHAVMGRTLSMTLKHLD SYLADCYDAIAVFLCIHIVLRFRNIAAKRDVPAL

25 DRYWGTGACLAMATV (SEQ ID NO:817). Moreover, fragments and variants of  
 these polypeptides (such as, for example, fragments as described herein, polypeptides  
 at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these  
 polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under  
 stringent conditions, to the polynucleotide encoding these polypeptides ) are  
 30 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 also encompassed by the invention.

The translation product of this gene shares sequence homology with a suppressor of actin mutation which is thought to be important in mutation suppression.

This gene is expressed primarily in fetal liver.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatic or metabolic conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
10 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver or cancer, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, metabolic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another  
15 tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 335 as residues: Val-53 to Arg-60, Thr-88 to Thr-94, Ala-142 to Ser-150, Gly-  
20 188 to Glu-196, Gly-208 to Ser-214, Thr-227 to Gly-232, Lys-279 to Phe-285.

The tissue distribution in liver, combined with the homology to a highly conserved suppressor of actin mutation, suggest that the protein product of this gene is useful for diagnosis and treatment of liver disorders or cancer. Similarly, the protein product of this gene is useful for the detection and treatment of  
25 hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells. In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various would-healing models and/or tissue trauma. Protein, as well as, antibodies directed  
30 against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:97 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1971 of SEQ ID NO:97, b is an integer of 15 to 1985, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:97, and where b is greater than or equal to a + 14.

#### *FEATURES OF PROTEIN ENCODED BY GENE NO: 88*

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

YEGKEFDYVFSIDVNEGGPSYKLPYNTSDDPWLTAYNFLQKNDLNPMFLDQ  
 VAKFIIDNTKGQMLGLGNPSFSDPFTGGGRYVPGSSGSSNTLPTADPFTGAGR  
 YVPGSASMGTTMAGVDPFTGNSAYRSAASKTMNIYFPKKEAVTFDQANPTQI  
 LGKLELNGTAPEEKKLTEDDLILLEKILSLICNSSSEKPTVQQLQILWKAINCP  
 EDIVFPALDILRLSIKHPVSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLAL  
 RTFCNCFVGQAGQKLMMSQRESLMSHAIELKSGSNKNI (SEQ ID NO:818),  
 HIALATL ALNYSVCFHKD (SEQ ID NO:819),  
 HNIEGKAQCLSLISTILEVVQDLEATFRLLVALGTLISDDSNVQLAKS (SEQ  
 ID NO:820), LGVDSQIKKYSSVSEPAKVSECCRIFILNLL (SEQ ID NO:821),  
 YEGKEFDYVFSIDVNEGGPSYKLPYNTSDDPWLTAYNFLQKNDLNPMFLDQ  
 VAKFIIDNTKGQMLGLGNPSFSDPFTGGGRYVPGSSGSSNTLPTADPFTGAGR  
 YVPGSASMGTTMAGVDPFTGNSAYRSAASKTMNIYFPKKEAVTFDQANPTQI  
 LGKLELNGTAPEEKKLTEDDLILLEKILSLICNSSSEKPTVQQLQILWKAINCP  
 EDIVFPALDILRLSIKHPVSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLAL  
 RTFCNCFVGQAGQKLMMSQRESLMSHAIELKSGSNKNIHIALATLALNYSVC  
 FHKDHNIEGKAQCLSLISTILEVVQDLEATFRLLVALGTLISDDSNVQLAKSL

GVDSQIKKYSSVSEPAKVSECCRFILNLL (SEQ ID NO:822),  
 LNLLLITQKVKCWDLGIPAFQIHLQVVVG (SEQ ID NO:823),  
 IKHPSVNENFCNEKEGAQFSSHLINLLNP (SEQ ID NO:824),  
 AIELKSGSNKNIHIALATLALN (SEQ ID NO:825),

- 5 VQLAKSLGVDSQIKKYSSVSEPA (SEQ ID NO:826),  
 YEGKEFDYVFSIDVNEGGPSYKLPYN (SEQ ID NO:827),  
 AYNFLQKNDLNPMFLDQVAK FIIDNT (SEQ ID NO:828),  
 SFSDPFTGGGRYVPG (SEQ ID NO:829), TADPFTGAGRY (SEQ ID NO:830),  
 TTMAGVDPFTGNSAYRSAA (SEQ ID NO:831), NIYFPKKEA (SEQ ID NO:832),  
 10 TFDQANPTQILGKLKELNG (SEQ ID NO:833),  
 PEDIVFPALDILRLSIKHPSVNENFCNEKE (SEQ ID NO:834),  
 QFSSHLINLLNPKG KPANQLLALRTFCNCFV (SEQ ID NO:835), and/or  
 QAGQKLMMSQRESLM SHAIELKSGSN (SEQ ID NO:836). Moreover, fragments  
 and variants of these polypeptides (such as, for example, fragments as described  
 15 herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%  
 identical to these polypeptides and polypeptides encoded by the polynucleotide which  
 hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 20 these polypeptides are also encompassed by the invention.

These polypeptides share significant homology with phospholipase A2  
 activating protein, which is thought to be important in signal transduction (see, e.g.,  
 Wang et al., Gene 161(2):237-241 (1995)). The gene encoding the disclosed cDNA is  
 believed to reside on chromosome 9. Accordingly, polynucleotides related to this  
 25 invention are useful as a marker in linkage analysis for chromosome 9.

This gene is expressed primarily in endothelial cells, to a less extent in  
 placenta, endometrial stromal cells, osteosarcoma, testis tumor, muscle, and infant  
 brain that are likely to be rich in blood vessels.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 30 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 disorders of the vascular system, aberrant angiogenesis, tumor angiogenesis, or

related disorders of endothelial tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system or tumors, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endothelial, placenta, skeletal, neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution of this gene in endothelial cells and several potential highly vascularized tissues, combined with the homology to the highly conserved phospholipase A2 activating protein suggest that this gene may be involved in transducing signals for endothelial cells in angiogenesis or vasculogenesis. Furthermore, the protein may show utility for the treatment, and/or prevention of embolism, thrombosis, aneurysm, atherosclerosis, microvascular disease, or stroke. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:98 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1402 of SEQ ID NO:98, b is an integer of 15 to 1416, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:98, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 89

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

YPNQDGDILRDQVLHEHIQRLSKVVTANHRALQIPEVYLREAPWPSAQSEIRT

5 ISAYKTPRDKVQCILRMCSTIMNLLSLANEDSVPGADDFVPVLVFLIKANPP  
CLLSTVQYISSFYASCLSGEESYWWMQFTA AVE (SEQ ID NO:837),

YPNQDGDILRDQVLHEHIQRLSKVVTANHRALQIPEVYLREAPWPSAQSEIRT

ISAYKTPRDKVQCILRMCSTIMNLLSLANEDSVPGADDFVPVLVFLIKANPP  
CLLSTVQYISSFYASCLSGEESYWWMQFTA AVEFIKTI (SEQ ID NO:838),

10 YPNQDGDILRDQVL (SEQ ID NO:839), EAPWPSAQSEI (SEQ ID NO:840),

PVLVFLIKANP (SEQ ID NO:845), SGEESYWWMQFTA AVEFIKTI (SEQ ID

NO:841), ADDFVPVLVFLIK ANPP (SEQ ID NO:842), YKTPRDKVQCIL (SEQ

ID NO:843), and/or GADDFVPV LVFVLIK (SEQ ID NO:844). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as

15 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides

of the invention are also encompassed by the invention. Polynucleotides encoding

20 these polypeptides are also encompassed by the invention.

The translation product of this gene shares sequence homology with human  
Ras inhibitor and yeast VPS9p which is thought to be important in Golgi vacuole  
transport. The gene encoding the disclosed cDNA is believed to reside on  
chromosome 9. Accordingly, polynucleotides related to this invention are useful as a  
25 marker in linkage analysis for chromosome 9.

This gene is expressed primarily in T cells and melanocytes.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
30 immune, hematopoietic, or integumentary disorders, such as dysfunctions and  
disorders involving T cells and melanocytes. Similarly, polypeptides and antibodies  
directed to these polypeptides are useful in providing immunological probes for

differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in T-cells and melanocytes, combined with the homology to a Ras inhibitor, indicates that the protein product of this gene is useful for regulating signal transduction; the diagnosis and treatment of disorders involving T cells and melanocytes, and potentially in the prevention or study of immune responses to aberrant integumentary cells and tissues, particularly in tumors and cancers, such as skin cancers. Moreover, the protein product of this gene is useful for the treatment, diagnosis, and/or prevention of various skin disorders including congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, Athlete's foot, and ringworm). Moreover, the protein product of this gene may also be useful for the treatment or diagnosis of various connective tissue disorders such as arthritis, trauma, tendonitis, chondromalacia and inflammation, autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal



chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:99 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1746 of SEQ ID NO:99, b is an integer of 15 to 1760, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:99, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 90**

The translation product of this gene shares sequence homology with neuronal olfactomedin-related ER localized protein which is thought to be important in the maintenance, growth, or differentiation of chemosensory cilia on the apical dendrites of olfactory neurons. Moreover, the protein also shares homology with the conserved human AMY protein which is thought to be a glial cell-specific transforming protein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

SARASTQPPAGQHGPC (SEQ ID NO:846),  
MPGRWRWQRDMHPARKLLSLLFLILMGTELTQD (SEQ ID NO:847),  
SAAPDSLLRSSKGSTRGSL (SEQ ID NO:848), AAIVIWRGKSESRIAKTPGI  
(SEQ ID NO:849), FRGGGTLVLPPTHTPEWLIL (SEQ ID NO:852),  
PLGITLPLGAPETGGGD (SEQ ID NO:850), NSARAS  
TQPPAGQHGPCMPGRWRWQRD (SEQ ID NO:853),  
YIVQGTTSFEMPTIPTPARHRAPHSPAGHVATAPQALHIKPAMHTAGRHAG  
CPSRSQ RHNPHRLFLEPPRAALCPKGG (SEQ ID NO:854),

ASNAHSWPARWLPFQVSAAQSPPPVSGAPKGSVMPKGRMSHSGVCVGGRTK  
 VPPPLKMPGVLAIRLSLFPLQMTIAAKDPLVLPFELLSRESGAAES (SEQ ID  
 NO:855), GRMSHSGVCVGGRTKVPPPLKMPGVLA (SEQ ID NO:856), and/or  
 CAAETWKGSQRAGQLCALLA (SEQ ID NO:851). Moreover, fragments and  
 5 variants of these polypeptides (such as, for example, fragments as described herein,  
 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
 under stringent conditions, to the polynucleotide encoding these polypeptides ) are  
 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 10 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
 9. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 9.

15 This gene is expressed in pineal gland.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 neurological and endocrine disorders. Similarly, polypeptides and antibodies directed  
 20 to these polypeptides are useful in providing immunological probes for differential  
 identification of the tissue(s) or cell type(s). For a number of disorders of the above  
 tissues or cells, particularly of the neurological or endocrine systems, expression of  
 this gene at significantly higher or lower levels may be routinely detected in certain  
 tissues or cell types (e.g., neural, endocrine, and cancerous and wounded tissues) or  
 25 bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or  
 another tissue or cell sample taken from an individual having such a disorder, relative  
 to the standard gene expression level, i.e., the expression level in healthy tissue or  
 bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID  
 30 NO: 338 as residues: Leu-20 to Ala-26, Arg-32 to Arg-39, Thr-104 to Gly-112.

The tissue distribution in pineal gland, combined with the homology to both  
 the olfactomedin-related, and AMY proteins, indicates that the protein product of this

gene is useful for maintenance, growth, or differentiation of neuron cells in pineal gland. Therefore, the protein product of this gene may be useful for the diagnosis and treatment of neurological disorders in pineal gland. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states,

5 behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive  
10 disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal  
15 differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:100 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
25 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 585 of SEQ ID NO:100, b is an integer of 15 to 599, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:100, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 91**

This gene is expressed primarily in prostate and apoptotic T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, immune, or hematopoietic disorders, particularly prostate disease and T cell dysfunction. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate cancer, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. prostate, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in prostate and T-cells indicates that the protein product of this gene is useful for the detection of abnormal activity in prostate and T cells, such as proliferative conditions of the prostate, or possibly treatment of this abnormality. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial

utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:101 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 770 of SEQ ID NO:101, b is an integer of 15 to 784, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:101, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 92**

The gene encoding the disclosed cDNA is believed to reside on chromosome  
20 19. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 19.

This gene is expressed primarily in prostate, and to a lesser extent, in smooth muscle cells, fibroblasts, and placenta.

Polynucleotides and polypeptides of the invention are useful as reagents for  
25 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders in prostate or vascular tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of  
30 the above tissues or cells, particularly of the prostate or vascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. prostate, musculoskeletal, cancerous and wounded tissues)

or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 340 as residues: Ser-38 to Lys-46.

The tissue distribution in prostate and smooth muscle indicates that the protein product of this gene is useful for regulating the function of prostate or highly vascularized tissues, such as the placenta. Similarly, the protein product of this gene may be useful in the treatment and/or detection of vascular disorders which include, but are not limited to, stroke, embolism, thrombosis, aneurysm, microvascular disease, or atherosclerosis. The protein may also show utility in the treatment or detection of proliferative disorders of the prostate or male reproductive system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:102 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 390 of SEQ ID NO:102, b is an integer of 15 to 404, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:102, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 93**

30 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: GHQTAPETPSRSD (SEQ ID NO:857). Moreover, fragments and variants of this polypeptide (such as, for

5

This gene is expressed primarily in embryos and fetal tissues, and to a lesser extent, in proliferative tissues.

10

25

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:103 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2204 of SEQ ID NO:103, b is an integer of 15 to 2218, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:103, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 94**

The translation product of this gene shares sequence homology with a transformation related protein which is thought to be important in transformation.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: SQTDR (SEQ ID NO:858). Polynucleotides encoding this polypeptides are also encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in female reproductive tissues, i.e., breast cancer cells, placenta, and ovary, and to a lesser extent, in fetal lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer or dysfunction of reproductive tissues, in addition to pulmonary or



developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproduction system, expression of this gene at

5 significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., pulmonary, reproductive, ovarian, breast, placental, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, pulmonary surfactant or sputum, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative

10 to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 342 as residues: Ser-50 to Pro-61.

The tissue distribution in female reproductive tissues, combined with the

15 homology to the transformation related protein, indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions caused by transformation, i.e. tumorigenesis in reproductive organs, (e.g. breast, placenta, and ovary). Similarly, expression within fetal tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of

20 cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein may also be useful in the treatment or detection of a

25 variety of pulmonary conditions, including, but not limited to emphysema, ARDS, cystic fibrosis, asthma, etc. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

30 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:104 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1337 of SEQ ID NO:104, b is an integer of 15 to 1351, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:104, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 95

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

NIYFKEKRKRGGAKMAGAIEN (SEQ ID NO:859),

VYLCAYTSTINVTVTANAKLINMCCLVDSNTRSCVVIDEGIFRSAEQFLIKFR

NKQSTIFPRFTWELHSIGLVFSIVFMGWCIQEHQSKDIQIPHPIDACEKGTVHL

DCDAAPFPMAFRYLTNDEEDDSHGSAGQGDKHEELEPKN (SEQ ID NO:860),

KMPCRMSPNSSIQVQSNPMENHSTGILIKVMEIPRAKMTFSRSTGGRDIMVILL

QYHTIMMKMLGVRKVFMANHTLVKPPFWWIPTNRISFISPIPTLIFFSFTGSR

MFKR (SEQ ID NO:861),

TTKSEKMQKSPWTFPWLTVMTHLLSGLKWPMKEYHGNSNAPSHLPRLQSM

RAVTMNVMSFLSWKLGLWPISFTF (SEQ ID NO:862),

IKFRNKQSTIFPRFTWELHSIGLVFSIVFMG (SEQ ID NO:863),

SSIQVQSNPMENHSTGILIKVMEIPRAKM (SEQ ID NO:864), and/or

LGVRKVFMANHTLVKPPFWWIPTNRISFISPIP (SEQ ID NO:865). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention. The gene encoding the disclosed cDNA is believed to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

5 This gene is expressed primarily in testes, rhabdomyosarcoma, infant brain and to a lesser extent in some tumors and highly vascularized tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, 10 tumorigenesis, abnormal angiogenesis, reproductive, vascular, and/or neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the tumor tissues or vascular tissues, expression of this gene at 15 significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., muscle, neural, developmental, vascular, reproductive, testicular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, seminal fluid, amniotic fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene 20 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 343 as residues: Arg-46 to Trp-54, Pro-60 to Ile-69, Asn-116 to Ala-122, Arg- 147 to Lys-153, Ser-158 to Glu-170, Ile-399 to Ser-405, Pro-486 to Met-499, Pro-502 25 to Asp-508.

The tissue distribution in infant brain indicates that the protein product of this gene is useful for a range of disease states including treatment of tumor or vascular disorders and the treatment of neurological disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, 30 obsessive compulsive disorder and panic disorder. Moreover, expression within vascular tissues indicates that the protein product of this gene is useful in the treatment and/or detection of a variety of vascular conditions, which include but are

not limited to emphysema, atherosclerosis, thrombosis, microvascular disease, stroke or aneurysm. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:105 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2052 of SEQ ID NO:105, b is an integer of 15 to 2066, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:105, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 96**

The translation product of this gene is homologous to the *Clostridium perfringens* enterotoxin (CPE) receptor gene product and shares sequence homology with a human ORF specific to prostate and a glycoprotein specific to oligodendrocytes, both of which are tissue specific proteins. See e.g., Katahira et al. J Cell Biol. 136(6):1239-1247 (1997). PMID: 9087440; UI: 97242441.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: TMA SMGLQV (SEQ ID NO:866),

KSWMMLWAVQDTGTITIRPANRNTTPATIMVLALALSSSRQLVHLPPTTDSST  
PRAATMMLMMTRARAACRSCGSASSES YTLHCIWPVLCTTQFIHRPSQMVCE  
VTMLLPKAVTRHMGSAQHSM T ASQPRTASAMPITCSPMEAI VQRPRELRT  
WKAEGIRLWGP (SEQ ID NO:867),

LQVMGIALAVLGWLAVMLCCALPMWRVT (SEQ ID NO:868),  
SNIVTSQTIWEGLWMNCVVQST (SEQ ID NO:869),  
QMQCKVYDSL LALPQDLQ (SEQ ID NO:870),

encompassed by the invention.

linkage analysis for chromosome 7.

to a lesser extent in several tumors and normal tissues.

having the disorder.

NO: 344 as residues: Gly-147 to Met-152, Cys-177 to Lys-188.

The tissue distribution in pancreas, combined with the homology to a prostate and oligodendrocyte-specific protein, indicates that the protein product of this gene is useful as a marker for the diagnosis or treatment of disorders in pancreas, ulcerative colitis, and tumors. Furthermore, identity to the human receptor for *Clostridium* perfringens enterotoxin indicates that the soluble portion of this receptor could be used in the treatment of food poisoning associated with *Clostridia* perfringens by blocking the activity of the perfringens enterotoxin. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:106 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1691 of SEQ ID NO:106, b is an integer of 15 to 1705, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:106, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 97**

The translation product of this gene shares sequence homology with an ATPase from *Saccharomyces cerevisiae* which is thought to be important in metabolism (See Genbank Accession No.g1181253).

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: PFTAIAGSEIFSLE (SEQ ID NO:874), SKTEALTQAFR (SEQ ID NO:875), VVHTVSLHEIDVINSRTQGFLALF (SEQ ID NO:876), PGVLFIDEVHMLDIE (SEQ ID NO:877),

AGIRQRFSARLWQLVSIMATVTATTKVPEIRDVTRIERIGAHSHIRGLGLDDAL  
 EPRQASQGMVGQLAARRAAGVVLEMIREGKIAGRAVLIAGQPGTGKTAIAM  
 GMAQALGPDTPFTAIAGSEIFSLEMSKTEALTQAFRRSIGVRIKEETEIEGEVV  
 EIQIDRPATGTGSKVVGKLTLLKTTMETIYDLGTKMIXSLTKDKVQAGDVITID  
 5 KATGKISKLGRSFTRARELRRYGLPDQVRAVPRWGAPETQGGGAHRVPARD  
 RRHQLSHPGLPGALLR (SEQ ID NO:878),

SPSTRRRARSPSWAAPSHAPANYDAMGSQTKFVQCPDGELQKRKEVVHTVS  
 LHEIDVINSRTQGFLALFSGDTGEIKSEVREQINAKVAEWREEGKAEIIPGVLF  
 DEVHMLDIESFSFLNRALES DMAPVQQVYGDAVRALVAGAPDSR DATVGG

10 VPNSCSPGDPLVLERPPPRWXS (SEQ ID NO:879),

WIPRAAGIRHEATNRGITRIRGTSYQSPHGIPIDLLDRRHVTLQGPVEEGEALD  
 VQHVDLVDEQHSRDDRLALLAPLSHLGIDLLTDF (SEQ ID NO:880),

YDAMGSQTKFVQCPDGELQKRKEVVHTVSL (SEQ ID NO:881),

KAEIIPGVLFIDEVHMLDIESFSFLNRALES (SEQ ID NO:882), and/or

15 EATNRGITRIRGTSYQSPHGIPIDLLDR (SEQ ID NO:883). Moreover, fragments  
 and variants of these polypeptides (such as, for example, fragments as described  
 herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%  
 identical to these polypeptides and polypeptides encoded by the polynucleotide which  
 hybridizes, under stringent conditions, to the polynucleotide encoding these  
 20 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testes and several hematopoietic cells.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 25 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 reproductive, immune, or hematopoietic disorders, particularly male infertility and  
 leukemia. Similarly, polypeptides and antibodies directed to these polypeptides are  
 useful in providing immunological probes for differential identification of the  
 30 tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,  
 particularly of the hematopoietic system, expression of this gene at significantly  
 higher or lower levels may be routinely detected in certain tissues or cell types (e.g.,

reproductive, immune, hematopoietic, testicular, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in testes and hematopoietic cells, combined with the homology to ATPases, indicates that the protein product of this gene is useful as a marker for the diagnosis and treatment of leukemia and other hematopoietic disorders. The protein may also show utility as a contraceptive, or for the treatment and/or detection of aberrant testicular function. The secreted protein can also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions and as nutritional supplements. It may also have a very wide range of biological activities. Typical of these are cytokine, cell proliferation/differentiation modulating activity or induction of other cytokines; immunostimulating/immunosuppressant activities (e.g. for treating human immunodeficiency virus infection, cancer, autoimmune diseases and allergy); regulation of hematopoiesis (e.g. for treating anemia or as adjunct to chemotherapy); stimulation or growth of bone, cartilage, tendons, ligaments and/or nerves (e.g. for treating wounds); stimulation of follicle stimulating hormone (for control of fertility); chemotactic and chemokinetic activities (e.g. for treating infections, tumors); hemostatic or thrombolytic activity (e.g. for treating hemophilia, cardiac infarction etc.); anti-inflammatory activity (e.g. for treating septic shock, Crohn's disease); as antimicrobials; for treating psoriasis or other hyperproliferative diseases; for regulation of metabolism, and behavior. Also contemplated is the use of the corresponding nucleic acid in gene therapy procedures. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:107 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is



cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1153 of SEQ ID NO:107, b is an integer of 15 to 1167, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:107, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 98

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MRSARPSLGCLPSWAFSQALNI (SEQ ID NO:884),  
 LLGLKGLAPAEISAVCEGNFN (SEQ ID NO:885),  
 VAHGLAWSYYIGYLRLILPELQARIR (SEQ ID  
 NO:886), TYNQHYNNLLRGAVSQRC (SEQ ID NO:887), ILLPLDCGVDPNLS  
 MADPNIRFLDKLPQQTGDRAGIKDRVYSN (SEQ ID NO:888),  
 SIYELLENGQRAGTCVLEYATPLQTLFAMSQYSQAGFSGEDRLEQ (SEQ ID  
 NO:889),  
 AKLFCRTLEDILADAPESQNNCRLLIAYQEPADDSSFSLSQEVLRLHLRQEEKEE  
 VTVGSLKTSAPVSTSTMSQEPPELLISGMEKPLPLRTDFS (SEQ ID NO:890),  
 LRLHSEKLPLAARSAGPSLLVIIQSSQCPGGRRYRGSYWRTVRACLGCPLRRG  
 ALLLSIYFYYSPLNAVGPFTW (SEQ ID NO:892),  
 VWLTPTFASWINCPSRPVTVLASRIGFTATASMSFWRTGSGRAPVSWSTPPPC  
 RLCLPCHNTVKLALAGRIGLSRPNSSAGHLRTSWQMPLSLRTTAASLPTRNLQ  
 MTAASRCPRRFSGTCGRRKRKRLWAA (SEQ ID NO:893),  
 GVCQVSFMGSPRPTPHPSPLPLPGDAELSQWYQQAPSPSGSWSCSIIGEPQQK  
 NGEETEEAEFGVLNPPAPTLLQHQGCYGLSCRATLA (SEQ ID NO:894), and/or  
 LLGLKGLAPAEISAVCEKGNFNVAHGLAWSYYIGYLRLILPEL (SEQ ID  
 NO:891). Moreover, fragments and variants of these polypeptides (such as, for  
 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.

Polynucleotides encoding these polypeptides are also encompassed by the invention.

5 This gene is expressed primarily in prostate BPH, and to a lesser extent, in bone marrow.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, hematopoietic, or immune disorders, particularly benign prostatic hypertrophy, prostate cancer, or leukemia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male urinary system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, hematopoietic, immune, prostatic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 346 as residues: Ile-60 to Asn-69, Leu-106 to Asp-112, Glu-130 to Gly-136, Phe-160 to Glu-167, Pro-184 to Cys-190, Glu-197 to Ser-202, Arg-215 to Glu-221, Thr-237 to Pro-242.

25 The tissue distribution in prostate tissue indicates that the protein product of this gene is useful for the diagnosis or treatment of reproductive disorders, such as benign prostatic hypertrophy or prostate cancer. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may

also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:108 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1893 of SEQ ID NO:108, b is an integer of 15 to 1907, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 99**

The gene encoding the disclosed cDNA is believed to reside on chromosome 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

This gene is expressed primarily in salivary gland.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, metabolic disorders, particularly of the salivary gland. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of glandular tissues, expression of

5 this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. salivary gland, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, chyme, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 The tissue distribution in salivary glands indicates that the protein product of this gene is useful for the treatment and/or detection of disorders of or injuries to the salivary gland or other glandular tissue. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:109 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 597 of SEQ ID NO:109, b is an integer of 15 to 611, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:109, and where b is greater than or equal to a + 14.

#### 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 100

30 The translation product of this gene shares sequence homology with a C.elegans gene. Based upon its degree of conservation, an important cellular function can be attributed to this protein. When tested against Jurkat cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates T-cells through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-

STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

- 5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 DPRVRLNSLTCKHIFISLTQ (SEQ ID NO:902),  
 TMKLLKLRNIVKLSLYRHFTN (SEQ ID NO:895),  
 TLILAVAASIVFIIWTTMKFRI (SEQ ID NO:896),  
 10 VTCQSDWRELWVDDAIWRLLFISMILFVI (SEQ ID NO:897),  
 MVLWRPSANNQRFAFSPLSEEEEEDEQ (SEQ ID NO:898),  
 MVLWRPSANNQRFAFSPLSEEEEEDEQ (SEQ ID NO:899),  
 KEPMLKESFEGMKMRSTKQEPNGNSKVNKAQEDDL (SEQ ID NO:900),  
 NAFGRHSTAVK (SEQ ID NO:903),  
 15 ESCLLCGISEYPIQRXICPGCFDPCRXAFSSETLTGSNPGHHSQSGIWHRQATP  
 GVTLHKVVVAXALYLLFSGMEGVLRVTGAQTDLASLAFIPLAFLDTALCWW  
 IFISLTQTMKLLKLRNIVKLSLYRHFTNTLILAVAASIVFIIWTTMKFRIVTCQ  
 SDWRELWVDDAIWRLLFISMILFVIMVLWRPSANNQRFAFSPLSEEEEEDEQK  
 EPMLKESFEGMKMRSTKQEPNGNSKVNKAQEDDLKWVEENVPSVTDVALP  
 20 ALLDSDEERMITHFERSKME (SEQ ID NO:904), and/or  
 KWVEENVPSVTDVALPALDSDEERMITHFERSKME (SEQ ID NO:901).  
 Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
 25 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention.  
 Polynucleotides encoding these polypeptides are also encompassed by the invention.
- The gene encoding the disclosed cDNA is believed to reside on chromosome  
 30 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

This gene is expressed primarily in thyroid, and to a lesser extent, in osteoclastoma, kidney medulla, and lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endocrine disorders, particularly thyroid dysfunction or cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., endocrine, skeletal, urogenital, renal, pulmonary, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, pulmonary surfactant or sputum, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 348 as residues: Lys-107 to Leu-124, Glu-150 to Thr-159, Pro-173 to Asp-179, Ser-192 to Ser-201.

The tissue distribution in thyroid, combined with the detected GAS biological activity, indicates that the protein product of this gene is useful for the diagnosis and treatment of thyroid dysfunction or cancer. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:110 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2618 of SEQ ID NO:110, b is an

integer of 15 to 2632, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:110, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 101

The gene encoding the disclosed cDNA is thought to reside on chromosome 16. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 16.

10 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: YEPMDFXMALIYD (SEQ ID NO:905), IRHELTVLRDT RPACA (SEQ ID NO:906), and/or MDFXMAL IYD (SEQ ID NO:907). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein,  
15 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are  
20 also encompassed by the invention.

This gene is expressed primarily in kidney cortex, and to a lesser extent, in adult brain, corpus colosum, hippocampus, and frontal cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
25 and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders, kidney disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system and renal system,  
30 expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. kidney, brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal

fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in adult brain, corpus colosum, hippocampus, and frontal cortex indicates that the protein product of this gene is useful for treatment or diagnosis of neurological disorders, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Furthermore, The tissue distribution in kidney indicates that this gene or gene product could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:111 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2235 of SEQ ID NO:111, b is an integer of 15 to 2249, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:111, and where b is greater than or equal to a + 14.



## FEATURES OF PROTEIN ENCODED BY GENE NO: 102

The translation product of this gene shares sequence homology with F15C11.2 of *C. elegans* which is of unknown function.

- 5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:
- MQEMMRNQDRALSNLESIPGGYNA (SEQ ID NO:908),  
 LRRMYTDIQEPMLSAAQEQQFGGNPF (SEQ ID NO:909),  
 ASLVSNTSSGEGSQPSRTENRDPLPNPWAPQT (SEQ ID NO:910),  
 10 SQSSSASSGTASTVGGTTGSTASGTSGQSTTAPNLVPGVGASMFNTPGMQSL  
 QQITENPQLMQNMLSAPY (SEQ ID NO:911),  
 MRSMMQSLSQNPDLAAQMMLNPLFAGNPQLQEQMRQQLPTFLQQ (SEQ  
 ID NO:912),  
 MQNPDTLSAMSNPRAMQALLQIQQLQTLATEAPGLIPGFTPGLGALGSTGG  
 15 SSGTNGSNATPSENTSPTAGT (SEQ ID NO:913),  
 TEPGHQQFIQQMLQALAGVNPQLQNPEVRFQQQLEQLSAMGFLNREANLQA  
 LIATGGDINAAIERLLGSQPS (SEQ ID NO:914),  
 RNPAMMQEMMRNQDRALSNLESIPGGYNALRRMYTDIQEPMLSAA (SEQ ID  
 NO:915), GNPFASLVSNTSS (SEQ ID NO:916), ENRDPLPNPWA (SEQ ID  
 20 NO:917), GKILKDQDTLSQHGIHD (SEQ ID NO:918), GLTVHLVIKTQNR  
 P (SEQ ID NO:919), SELQSQMQRQLLSNPPEMM (SEQ ID NO:920),  
 PEISHMLNPNPDIMR (SEQ ID NO:921), and/or RQLIMANPQMQLIQNRNP (SEQ  
 ID NO:922). Moreover, fragments and variants of these polypeptides (such as, for  
 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 25 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides ) are encompassed by the invention. Antibodies that  
 bind polypeptides of the invention are also encompassed by the invention.  
 Polynucleotides encoding these polypeptides are also encompassed by the invention.
- 30 This gene is expressed primarily in breast.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include, but are not limited to, breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of tumor systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. breast, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in breast indicates that the protein product of this gene is useful for treatment and diagnosis of some types of breast cancer. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:112 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2184 of SEQ ID NO:112, b is an integer of 15 to 2198, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:112, and where b is greater than or equal to a + 14.

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 103**

The translation product of this gene shares sequence homology with secreted serine proteases and lysozyme C precursor, which is thought to be important in bacteriolytic function.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

NLCHVDCQDLLNP NLLAGIHCAKRIVS (SEQ ID NO:923),

LDGFEGYSLSDWLCLAFVESKFN (SEQ ID NO:924),

5 NENADGSFDYGLFQINSHYWCN (SEQ ID NO:925),

NLCHVDCQDLLNP NLLAGIHCAKRIVS (SEQ ID NO:926), and/or

EPSALSCTSSPPR (SEQ ID NO:927). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides

10 and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, infection, immune system disorders, reproductive disorders. Similarly, polypeptides

20 and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. testes, cancerous and

25 wounded tissues) or bodily fluids (e.g., lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

30 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 351 as residues: Ile-62 to Phe-70, Asn-78 to Asn-84.

The tissue distribution in testes, combined with the homology to lysozyme C precursor indicates that the protein product of this gene is useful for boosting the monocyte-macrophage system, and for enhancing the activity of immune agents. Alternatively, the tissue distribution indicates that the protein product of this gene is useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:113 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1029 of SEQ ID NO:113, b is an integer of 15 to 1043, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 104**

This gene is expressed primarily in apoptotic T-cell, and to a lesser extent in CD34(+) cells..

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders. Similarly, polypeptides and antibodies directed to these

5 polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, 10 serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in T-cells indicates that the protein product of this gene 15 is useful for treatment and diagnosis of some immune disorders. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the 20 protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion 25 of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed 30 tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:114 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 689 of SEQ ID NO:114, b is an integer of 15 to 703, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:114, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 105

The translation product of this gene shares sequence homology with ARI protein of Drosophila (See Genbank Accession 2058299; EMBL: locus DMARIADNE, accession X98309), which is thought to be important in axonal path-finding in the central nervous system.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

IREVNEVIQNPAT (SEQ ID NO:928),

ITRILLSHFNWDKEKLMERYFDGNLEKLFA (SEQ ID NO:929),

NTRSSAQDMPCQICYLNYPNSYF (SEQ ID NO:930), TGL

ECGHKFCMQCWSEYLTTKIMEEGMGQTISCPAHG (SEQ ID NO:936),

CDILVDDNTVMRLITDSKVKLKYQHLITNSFVECNRLKWCAPDCHHVVKV

QYPDAKPV (SEQ ID NO:931),

CDILVDDNTVMRLITDSKVKLKYQHLITNSFVECNRLKWCAPDCHHVVKV

(SEQ ID NO:932),

GCNHMVCRNQNKAEFCWVCLGPWEPHGSAWYNCNRYNEDDAKAARDA

QERSRAALQRYL (SEQ ID NO:933),

FYCNRYMNHMQSLRFEHKLYAQVKQKMEEMQQHNMSWIEVQFLKKAQDV

LCQCRATLMT (SEQ ID NO:934), and/or

YVFAFYLLKNNQSIIFENNQADLENATEVLSGYLERDISQDSLQDIKQKVQDK

YRYCESR (SEQ ID NO:935). Moreover, fragments and variants of these

polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in adult brain, and to a lesser extent in testes, endometrial tumor, melanocytes, and infant brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases or injuries involving axonal path development. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, testes, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in adult brain, combined with the homology to ARI protein indicates that the protein product of this gene is useful for the treatment of disease states or injuries involving axonal path development, including neurodegenerative diseases and nerve injury, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well

as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:115 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3670 of SEQ ID NO:115, b is an integer of 15 to 3684, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:115, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 106**

The translation product of this gene shares sequence homology with cytochrome b561 [*Sus scrofa*] which is thought to be an integral membrane protein of neuroendocrine storage vesicles of neurotransmitters and peptide hormones. The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

This gene is expressed primarily in frontal cortex, and to a lesser extent in rhabdomyosarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell



types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 354 as residues: Ser-18 to Pro-24.

The tissue distribution in frontal cortex, combined with the homology to cytochrome b561 [*Sus scrofa*] indicates that the protein product of this gene is useful for the treatment and diagnosis of neurological disorders. This gene may also be important in the regulation of some types of cancers. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of disorders of the brain and nervous system. Elevated expression of this gene product within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:116 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1951 of SEQ ID NO:116, b is an integer of 15 to 1965, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:116, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 107

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MWGYLFVDAAWNFLGCLICGW (SEQ ID NO:937),

MHFISSGNVSAIRSSILLRLXSLSYLGNCRLVSAIFVYFLLFLLLS (SEQ ID

5 NO:938), and/or

MDQALRGSPSEGFSTDPSPQVGRQIPSFPPWRRLVLPKASGCFLEREWLVCV

FKLRTRPGAEAHAYNSSILGGRGKGIT (SEQ ID NO:939). Moreover, fragments

and variants of these polypeptides (such as, for example, fragments as described

herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%

10 identical to these polypeptides and polypeptides encoded by the polynucleotide which

hybridizes, under stringent conditions, to the polynucleotide encoding these

polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides

of the invention are also encompassed by the invention. Polynucleotides encoding

these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in pancreas tumor, and to a lesser extent in cerebellum.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,

20 pancreatic tumors. Similarly, polypeptides and antibodies directed to these

polypeptides are useful in providing immunological probes for differential

identification of the tissue(s) or cell type(s). For a number of disorders of the above

tissues or cells, particularly of the endocrine system, expression of this gene at

significantly higher or lower levels may be routinely detected in certain tissues or cell

25 types (e.g. pancreas, cancerous and wounded tissues) or bodily fluids (e.g., lymph,

serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample

taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an

individual not having the disorder.

30 Predicted epitopes include those comprising a sequence shown in SEQ ID

NO: 355 as residues: Pro-22 to Phe-33.

The tissue distribution in pancreas tumors indicates that the protein product of this gene is useful for diagnosis and treatment of pancreatic tumors, and/or tumors of metabolic tissues and cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:117 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 489 of SEQ ID NO:117, b is an integer of 15 to 503, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:117, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 108

The gene encoding the disclosed cDNA is thought to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MLPALASCCHFSPPEQAARLKKLQEKEKQKVEFRKRMEKEVSDFIQDSGQI  
KKKFQPMNKIERSILHDVVEVAGLTSFSFGEDDDCRYVMIFKKEFAPSDEELD  
SYRRGEEWDPQKAEEKRNXXKELAQRR (SEQ ID NO:940),  
EEEAQQGPVVVSPASDYKDKYSHLIGKGAAKDAAHMLQANKTYGDXPVA  
NKRDRSIEEAMNEIRAKKRLRQSGE (SEQ ID NO:941),  
PPRRPAQLPLTPGAGQGAGRDKAAAIRAHGAPPLNHLIP (SEQ ID NO:942),  
AVPQAGGKQVFDLSPLELGYVRGMCVCV (SEQ ID NO:943) and/or  
MLPALASCCHFSPPEQAARLKKLQEKEKQKVEFRKRMEKEVSDFIQDSGQI

KKKFQPMNKIERSILHDVVEVAGLTSFSFGEDDDCRYVMIFKKEFAPSDEELD  
SYRRGEEWDPQKAEEKRNXXKELAQRQEEEEAAQGPVVVSPASDYKDKYSHL  
IGKGAAKDAAHMLQANKTYGCXPVANKRDTRSIEEAMNEIRAKKRLRQSGE

(SEQ ID NO:944). Moreover, fragments and variants of these polypeptides (such as,  
5 for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides ) are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
10 invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

The translation product of this gene shares sequence homology with FSA-1,  
which may play a role as a structural protein component of the acrosome. The  
mammalian spermatozoon undergoes continuous modifications during  
15 spermatogenesis, maturation in the epididymis, and capacitation in the female  
reproductive tract. Only the capacitated spermatozoa are capable of binding the zona-  
intact egg and undergoing the acrosome reaction. The fertilization process is a net  
result of multiple molecular events which enable ejaculated spermatozoa to recognize  
and bind to the egg's extracellular coat, the zona pellucida (ZP). Sperm-egg  
20 interaction is a species-specific event which is initiated by the recognition and binding  
of complementary molecule(s) present on sperm plasma membrane (receptor) and the  
surface of the ZP (ligand). This is a carbohydrate-mediated event which initiates a  
signal transduction cascade resulting in the exocytosis of acrosomal contents. This  
step is believed to be a prerequisite which enables the acrosome reacted spermatozoa  
25 to penetrate the ZP and fertilize the egg. Recently, another group published this gene,  
calling it sperm acrosomal protein [Homo sapiens] (Proc. Natl. Acad. Sci. U.S.A.  
95 (14), 8175-8180 (1998)).

This gene is expressed primarily in fetal kidney and sperm.

Polynucleotides and polypeptides of the invention are useful as reagents for  
30 differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
male reproductive disorders, especially involving acrosomal dysfunction. Similarly,

polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. sperm, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 356 as residues: Met-12 to Gln-30, Lys-35 to Val-46, Arg-49 to Val-56, Gln-61 to Glu-77, Gly-96 to Cys-101, Glu-110 to Lys-139, Leu-141 to Gln-151, Ser-161 to Tyr-167, Asn-196 to Ile-203, Arg-211 to Ser-227.

The tissue distribution in sperm, combined with the homology to FSA-1 and the Homo sapiens sperm acrosomal protein indicates that the protein product of this gene is useful for the treatment of infertility due to acrosomal dysfunction of sperm. Protein may also be useful as a contraceptive either alone, or in combination with other therapies. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:118 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1057 of SEQ ID NO:118, b is an integer of 15 to 1071, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:118, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 109**

This gene is expressed primarily in pituitary tissue, and to a lesser extent in epididymus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, male reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. epididymus, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 357 as residues: Met-1 to Trp-6.

Because the gene is found in both pituitary and epididymus, this indicates that the protein product of this gene is useful for the treatment and diagnosis of male reproductive disorders. This may involve a secreted peptide produced in the pituitary targeting the epididymus. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:119 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1087 of SEQ ID NO:119, b is an

integer of 15 to 1101, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:119, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 110

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

LLCPVLNSGXSWNFPHPHQPEYSFHGFHSTRLWI (SEQ ID NO:945), and/or

10 PSTPWFLFLLGLTCPFSTSHPRWDSIPP (SEQ ID NO:946). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these  
15 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in resting T-cells. .

Polynucleotides and polypeptides of the invention are useful as reagents for  
20 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, T-cell disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,  
25 particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
30 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in T-cells indicates that the protein product of this gene is useful for the treatment and diagnosis of certain immune disorders, especially those involving T-cells. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:120 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 268 of SEQ ID NO:120, b is an integer of 15 to 282, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:120, and where b is greater than or equal to a + 14.

### 30 FEATURES OF PROTEIN ENCODED BY GENE NO: 111



The gene encoding the disclosed cDNA is thought to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in cerebellum and whole brain, and to a lesser extent in infant brain and fetal kidney.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 359 as residues: Asp-48 to Gly-55.

The tissue distribution in cerebellum and whole brain indicates that the protein product of this gene is useful for diagnosis and treatment of neurological disorders, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:121 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
 5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2621 of SEQ ID NO:121, b is an integer of 15 to 2635, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:121, and where b is greater than or equal to a + 14.

10

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 112**

The translation product of this gene shares sequence homology with yeast mitochondrial ribosomal protein, which is homologous to ribosomal protein s15 of  
 15 *E.coli*, which is thought to be important in the early assembly of ribosomes (See Genbank Accession No. M38016). The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in developmental tissues.

20

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, development of cancers and tumors in addition to healing wounds. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing  
 25 immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and developmental systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. developmental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine,  
 30 synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in developmental tissues, combined with the homology to ribosomal protein s15 of E. coli indicates that the protein product of this gene is useful for the diagnosis and/or treatment of diseases related to the assembly of ribosomes in the mitochondria, which is important in the translation of RNA into protein. Therefore, this indicates that the protein product of this gene is also useful for the diagnosis and intervention of multiple tumors, as well as in healing wounds, which are thought to be under similar regulation as developmental tissues. Protein, as well as, antibodies directed against the protein have utility as tumor markers, in addition to immunotherapy targets, for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:122 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 980 of SEQ ID NO:122, b is an integer of 15 to 994, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:122, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 113**

For purposes of this application, this gene and its corresponding translation product are known as the B7-H4 gene and B7-H4 protein. This protein is believed to reside as a cell-surface molecule, and the transmembrane domain of this protein is believed to embody the following preferred amino acid residues:

GIVAFIVFLLLIMLIFL (SEQ ID NO: 1236). Polynucleotides encoding this polypeptide are also encompassed by the invention, as are antibodies that bind the polypeptide. The B7-H4 gene shares sequence homology with members of the B7

family of ligands (i.e., B7-1 (See Genbank Accession 507873)). These proteins and their corresponding receptors play vital roles in the growth, differentiation and death of T cells. For example, some members of this family (i.e., B7-H1) are involved in costimulation of the T cell response, as well as inducing increased cytokine

production. Therefore, agonists and/or antagonists such as antibodies or small molecules directed against the B7-H4 gene are useful for treating T cell mediated immune system disorders. The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention have uses, such as, for example, as a marker in linkage analysis for chromosome 1.

The translation product of this gene shares sequence homology with human poliovirus receptor precursors which are thought to be important in viral binding and uptake. The translation product of this gene also shares homology with a mouse member of the immunoglobulin superfamily, which is thought to be important in proper immune function (GENBANK: accession AF061260).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

ELISISNVALADEGEYTCSTFTMPVRTAKSLVTVLGIPQKPITGYKSSLREKD  
TATLNCQSSGSKPAARLTWRKGDQELHGEPTRIQEDPNGKTFTVSSSVTFQVT  
REDDGASIVCSVNHESLKGADRSTSQRIVLYTPTAMIRPDPPHPREGQKLLL

HCEGRGNPVPQQYLWEKEGSVPPLKMTQESALIFPFLNKSDSGTYGCTATSN  
MGSYKAYYTLNVND (SEQ ID NO:947),

ELISISNVALADEGEYTCSTFTMPVRTAKSLVTVLGIPQKPITGYKSSLREKD  
TATLNCQSS (SEQ ID NO:948),

CQSSGSKPAARLTWRKGDQELHGEPTRIQEDPNGKTFTVSSSVTFQVTREDD

GASIVCSVNHESL (SEQ ID NO:949),

HESLKGADRSTSQRIVLYTPTAMIRPDPPHPREGQKLLHCEGRGNPVPQQY  
LWEKE (SEQ ID NO:950),

WEKEGSVPPLKMTQESALIFPFLNKSDSGTYGCTATSNMGSYKAYYTLNVND  
(SEQ ID NO:951), PSPVPSSSSTYHAIIGGIVAFIVFLLLIMLIFLGHY (SEQ ID

NO:952), and/or LIRHKGTYLTAEAKGSDDAPDADTAIINAEAGGQSGGDDKK

EYFI (SEQ ID NO:953). Moreover, fragments and variants of these polypeptides

(such as, for example, fragments as described herein, polypeptides at least 80%, 85%,

90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

A splice variant of this gene has been identified which encodes a polypeptide lacking the following amino acid segment of SEQ ID NO: 361:

DGYWQEQLDELGLAPLDEAISSTWSSPDMLASQ (SEQ ID NO: 1240). This splice variant was identified in clone HCE1K47, deposited in ATCC Deposit Accession No. PTA-2574 on October 5, 2000 and in ATCC Deposit Accession No. PTA-3070 on February 16, 2001.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

NLSQDGYWQEQLDELGLAPLDEAISSTWSSPDMLASQDSQP (SEQ ID NO: 1241), DGYWQEQLDELGLAPLDEAISSTWSSPDMLASQ (SEQ ID NO: 1240), and/or NLSQDSQP (SEQ ID NO: 1242). In a further specific embodiment, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

MGAPAASLLLLLLFACCWAPGGANLSQDDSQPWTSDET VVAGGT VVLKCQ VKDHEDSSLQWS (SEQ ID NO: 1243). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been discovered that this gene is expressed almost exclusively in human brain tissue.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve,

thirteen, fourteen, fifteen, sixteen, or all sixteen of the immunogenic epitopes of the extracellular portion of the B7-H4 protein shown in SEQ ID NO: 361 as residues:

Leu-26 to Asp-36, Gln-63 to Asp-71, Lys-87 to Gln-102, Gly-107 to Arg-116, Tyr-172 to Ala-182, Thr-198 to His-207, Glu-209 to Lys-220, Thr-233 to Gly-238, Glu-248 to Gln-259, Pro-273 to Gln-282, Glu-289 to Gln-297, Asn-324 to Thr-330, Val-350 to Pro-355, Ile-390 to Thr-395, Ala-401 to Ala-410, Glu-418 to Tyr-430.

Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these peptides.

In additional nonexclusive embodiments, polypeptides of the invention comprise, or alternatively consist of, one or more of the following amino acid sequences:

1.) The extracellular domain of the B7-H4 protein:

MGAPAASLLLLLLFACCWAPGGANLSQDGYWQEQLGTLAPLDEAISST  
WSSPDMLASQDSQPWTSDETVVAGGTVVLKCQVKDHEDSSLQWSNPAQQT  
LYFGEKRALRDNRIQLVTSTPHELSSISNVALADEGEYTCSTFTMPVRTAKSL  
VTVLGIPQKPIITGYKSSLREKDTATLNCQSSGSKPAARLTWRKGDQELHGEP  
TRIQUEDPNGKTFTVSSSVTFQVTRDDGASIVCSVNHESLKGADRSTSQRIVL  
YTPTAMIRPDPPHPREGQKLLHCEGRGNPVPQQYLWEKEGSVPPLKMTQES  
ALIFPFLNKSDSGTYGCTATSNMGSYKAYYTLNVNDPSPVPSSSSTYHAIIG

(SEQ ID NO: 1237);

2.) The mature extracellular domain of the B7-H4 protein:

NLSQDGYWQEQLGTLAPLDEAISSTVWSSPDMLASQDSQPWTSDETVV  
AGGTVVLKCQVKDHEDSSLQWSNPAQQTLYFGEKRALRDNRIQLVTSTPHEL  
SSISNVALADEGEYTCSTFTMPVRTAKSLVTVLGIPQKPIITGYKSSLREKDTA  
TLNCQSSGSKPAARLTWRKGDQELHGEPTRIQUEDPNGKTFTVSSSVTFQVTR  
DDGASIVCSVNHESLKGADRSTSQRIVLYTPTAMIRPDPPHPREGQKLLHCE  
GRGNPVPQQYLWEKEGSVPPLKMTQESALIFPFLNKSDSGTYGCTATSNMG  
SYKAYYTLNVNDPSPVPSSSSTYHAIIG (SEQ ID NO: 1238); and/or

3.) The anticipated leader sequence of the B7-H4 protein:

MGAPAASLLLLLLFACCWAPGGA (SEQ ID NO: 1239).

Polynucleotides encoding these polypeptides are also encompassed by the invention, as are antibodies that bind one or more of these polypeptides.

Also preferred are polypeptides comprising, or alternatively consisting of, fragments of the mature extracellular portion of the B7-H4 protein demonstrating functional activity (SEQ ID NO: 361). Polynucleotides encoding these polypeptides are also encompassed by the invention. By functional activity is meant, a polypeptide fragment capable of displaying one or more known functional activities associated with the full-length (complete) B7-H4 protein. Such functional activities include, but are not limited to, biological activity (e.g., T cell costimulatory activity, ability to bind ICOS, and ability to induce or inhibit cytokine production), antigenicity [ability to bind (or compete with a B7-H4 polypeptide for binding) to an anti-B7-H4 antibody], immunogenicity (ability to generate antibody which binds to a B7-H4 polypeptide), ability to form multimers with B7-H4 polypeptides of the invention, and ability to bind to a receptor or ligand for a B7-H4 polypeptide.

Figures 3A-C show the nucleotide (SEQ ID NO: 123) and deduced amino acid sequence (SEQ ID NO: 361) corresponding to this gene.

Figure 4 shows an analysis of the amino acid sequence (SEQ ID NO: 361). Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings of the recited computer algorithms. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. Polypeptides comprising, or alternatively consisting of, domains defined by these graphs are contemplated by the present invention, as are polynucleotides encoding these polypeptides.

The data presented in Figure 4 are also represented in tabular form in Table 4. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figures 3A-3C, and Table 4: "Res": amino acid residue of SEQ ID NO: 361 and Figures 3A-3C; "Position": position of the corresponding residue within SEQ ID NO: 361 and Figures 3A-3C; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-

Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

5 Preferred embodiments of the invention in this regard include fragments that comprise, or alternatively consisting of, one or more of the following regions: alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions,  
10 alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions. The data representing the structural or functional attributes of the protein set forth in Figure 4 and/or Table 4, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data  
15 presented in columns VIII, IX, XIII, and XIV of Table 4 can be used to determine regions of the protein which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or XIV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in  
20 which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figure 4, but may, as shown in Table 4, be represented or identified by using tabular representations of the data presented in Figure 4. The DNA\*STAR computer algorithm used to generate  
25 Figure 4 (set on the original default parameters) was used to present the data in Figure 4 in a tabular format (See Table 4). The tabular format of the data in Figure 4 is used to easily determine specific boundaries of a preferred region.

The present invention is further directed to fragments of the polynucleotide sequences described herein. By a fragment of, for example, the polynucleotide  
30 sequence of a deposited cDNA or the nucleotide sequence shown in SEQ ID NO: 123, is intended polynucleotide fragments at least about 15nt, and more preferably at least about 20 nt, at least about 25nt, still more preferably at least about 30 nt, at least



about 35nt, and even more preferably, at least about 40 nt in length, at least about 45nt in length, at least about 50nt in length, at least about 60nt in length, at least about 70nt in length, at least about 80nt in length, at least about 90nt in length, at least about 100nt in length, at least about 125nt in length, at least about 150nt in length, at least about 175nt in length, which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 200-1500 nt in length are also useful according to the present invention, as are fragments corresponding to most, if not all, of the nucleotide sequence of a deposited cDNA or as shown in SEQ ID NO: 123. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of a deposited cDNA or the nucleotide sequence as shown in SEQ ID NO: 123. In this context "about" includes the particularly recited size, an sizes larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Representative examples of polynucleotide fragments of the invention include, for example, fragments that comprise, or alternatively, consist of, a sequence from about nucleotide 1 to about 50, from about 51 to about 100, from about 101 to about 150, from about 151 to about 200, from about 201 to about 250, from about 251 to about 300, from about 301 to about 350, from about 351 to about 400, from about 401 to about 450, from about 451 to about 500, and from about 501 to about 550, and from about 551 to about 600, from about 601 to about 650, from about 651 to about 700, from about 701 to about 750, from about 751 to about 800, and from about 801 to about 860, of SEQ ID NO: 123, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. In additional embodiments, the polynucleotides of the invention encode functional attributes of the corresponding protein.

Preferred polypeptide fragments of the invention comprise, or alternatively consist of, the secreted protein having a continuous series of deleted residues from the amino or the carboxyl terminus, or both. Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-432 where m is an integer from 2 to 426, where m corresponds to the position of the amino acid residue identified in SEQ ID NO: 361. More in particular, the invention provides

polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: G-2 to I-432; A-3 to I-432; P-4 to I-432; A-5 to I-432; A-6 to I-432; S-7 to I-432; L-8 to I-432; L-9 to I-432; L-10 to I-432; L-11 to I-432; L-12 to I-432; L-13 to I-432; L-14 to I-432; F-15 to I-432; A-16 to I-432; C-17 to I-432; C-18 to I-432; W-19 to I-432; A-20 to I-432; P-21 to I-432; G-22 to I-432; G-23 to I-432; A-24 to I-432; N-25 to I-432; L-26 to I-432; S-27 to I-432; Q-28 to I-432; D-29 to I-432; G-30 to I-432; Y-31 to I-432; W-32 to I-432; Q-33 to I-432; E-34 to I-432; Q-35 to I-432; D-36 to I-432; L-37 to I-432; E-38 to I-432; L-39 to I-432; G-40 to I-432; T-41 to I-432; L-42 to I-432; A-43 to I-432; P-44 to I-432; L-45 to I-432; D-46 to I-432; E-47 to I-432; A-48 to I-432; I-49 to I-432; S-50 to I-432; S-51 to I-432; T-52 to I-432; V-53 to I-432; W-54 to I-432; S-55 to I-432; S-56 to I-432; P-57 to I-432; D-58 to I-432; M-59 to I-432; L-60 to I-432; A-61 to I-432; S-62 to I-432; Q-63 to I-432; D-64 to I-432; S-65 to I-432; Q-66 to I-432; P-67 to I-432; W-68 to I-432; T-69 to I-432; S-70 to I-432; D-71 to I-432; E-72 to I-432; T-73 to I-432; V-74 to I-432; V-75 to I-432; A-76 to I-432; G-77 to I-432; G-78 to I-432; T-79 to I-432; V-80 to I-432; V-81 to I-432; L-82 to I-432; K-83 to I-432; C-84 to I-432; Q-85 to I-432; V-86 to I-432; K-87 to I-432; D-88 to I-432; H-89 to I-432; E-90 to I-432; D-91 to I-432; S-92 to I-432; S-93 to I-432; L-94 to I-432; Q-95 to I-432; W-96 to I-432; S-97 to I-432; N-98 to I-432; P-99 to I-432; A-100 to I-432; Q-101 to I-432; Q-102 to I-432; T-103 to I-432; L-104 to I-432; Y-105 to I-432; F-106 to I-432; G-107 to I-432; E-108 to I-432; K-109 to I-432; R-110 to I-432; A-111 to I-432; L-112 to I-432; R-113 to I-432; D-114 to I-432; N-115 to I-432; R-116 to I-432; I-117 to I-432; Q-118 to I-432; L-119 to I-432; V-120 to I-432; T-121 to I-432; S-122 to I-432; T-123 to I-432; P-124 to I-432; H-125 to I-432; E-126 to I-432; L-127 to I-432; S-128 to I-432; I-129 to I-432; S-130 to I-432; I-131 to I-432; S-132 to I-432; N-133 to I-432; V-134 to I-432; A-135 to I-432; L-136 to I-432; A-137 to I-432; D-138 to I-432; E-139 to I-432; G-140 to I-432; E-141 to I-432; Y-142 to I-432; T-143 to I-432; C-144 to I-432; S-145 to I-432; I-146 to I-432; F-147 to I-432; T-148 to I-432; M-149 to I-432; P-150 to I-432; V-151 to I-432; R-152 to I-432; T-153 to I-432; A-154 to I-432; K-155 to I-432; S-156 to I-432; L-157 to I-432; V-158 to I-432; T-159 to I-432; V-160 to I-432; L-161 to I-432; G-162 to I-432; I-163 to I-432; P-164 to I-432; Q-165 to I-432; K-166 to I-432; P-167 to I-432; I-168 to I-432; I-169 to I-432;

T-170 to I-432; G-171 to I-432; Y-172 to I-432; K-173 to I-432; S-174 to I-432; S-  
 175 to I-432; L-176 to I-432; R-177 to I-432; E-178 to I-432; K-179 to I-432; D-180  
 to I-432; T-181 to I-432; A-182 to I-432; T-183 to I-432; L-184 to I-432; N-185 to I-  
 432; C-186 to I-432; Q-187 to I-432; S-188 to I-432; S-189 to I-432; G-190 to I-432;  
 5 S-191 to I-432; K-192 to I-432; P-193 to I-432; A-194 to I-432; A-195 to I-432; R-  
 196 to I-432; L-197 to I-432; T-198 to I-432; W-199 to I-432; R-200 to I-432; K-201  
 to I-432; G-202 to I-432; D-203 to I-432; Q-204 to I-432; E-205 to I-432; L-206 to I-  
 432; H-207 to I-432; G-208 to I-432; E-209 to I-432; P-210 to I-432; T-211 to I-432;  
 R-212 to I-432; I-213 to I-432; Q-214 to I-432; E-215 to I-432; D-216 to I-432; P-217  
 10 to I-432; N-218 to I-432; G-219 to I-432; K-220 to I-432; T-221 to I-432; F-222 to I-  
 432; T-223 to I-432; V-224 to I-432; S-225 to I-432; S-226 to I-432; S-227 to I-432;  
 V-228 to I-432; T-229 to I-432; F-230 to I-432; Q-231 to I-432; V-232 to I-432; T-  
 233 to I-432; R-234 to I-432; E-235 to I-432; D-236 to I-432; D-237 to I-432; G-238  
 to I-432; A-239 to I-432; S-240 to I-432; I-241 to I-432; V-242 to I-432; C-243 to I-  
 15 432; S-244 to I-432; V-245 to I-432; N-246 to I-432; H-247 to I-432; E-248 to I-432;  
 S-249 to I-432; L-250 to I-432; K-251 to I-432; G-252 to I-432; A-253 to I-432; D-  
 254 to I-432; R-255 to I-432; S-256 to I-432; T-257 to I-432; S-258 to I-432; Q-259  
 to I-432; R-260 to I-432; I-261 to I-432; E-262 to I-432; V-263 to I-432; L-264 to I-  
 432; Y-265 to I-432; T-266 to I-432; P-267 to I-432; T-268 to I-432; A-269 to I-432;  
 20 M-270 to I-432; I-271 to I-432; R-272 to I-432; P-273 to I-432; D-274 to I-432; P-  
 275 to I-432; P-276 to I-432; H-277 to I-432; P-278 to I-432; R-279 to I-432; E-280  
 to I-432; G-281 to I-432; Q-282 to I-432; K-283 to I-432; L-284 to I-432; L-285 to I-  
 432; L-286 to I-432; H-287 to I-432; C-288 to I-432; E-289 to I-432; G-290 to I-432;  
 R-291 to I-432; G-292 to I-432; N-293 to I-432; P-294 to I-432; V-295 to I-432; P-  
 25 296 to I-432; Q-297 to I-432; Q-298 to I-432; Y-299 to I-432; L-300 to I-432; W-301  
 to I-432; E-302 to I-432; K-303 to I-432; E-304 to I-432; G-305 to I-432; S-306 to I-  
 432; V-307 to I-432; P-308 to I-432; P-309 to I-432; L-310 to I-432; K-311 to I-432;  
 M-312 to I-432; T-313 to I-432; Q-314 to I-432; E-315 to I-432; S-316 to I-432; A-  
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 30 I-432; L-323 to I-432; N-324 to I-432; K-325 to I-432; S-326 to I-432; D-327 to I-  
 432; S-328 to I-432; G-329 to I-432; T-330 to I-432; Y-331 to I-432; G-332 to I-432;  
 C-333 to I-432; T-334 to I-432; A-335 to I-432; T-336 to I-432; S-337 to I-432; N-

338 to I-432; M-339 to I-432; G-340 to I-432; S-341 to I-432; Y-342 to I-432; K-343 to I-432; A-344 to I-432; Y-345 to I-432; Y-346 to I-432; T-347 to I-432; L-348 to I-432; N-349 to I-432; V-350 to I-432; N-351 to I-432; D-352 to I-432; P-353 to I-432; S-354 to I-432; P-355 to I-432; V-356 to I-432; P-357 to I-432; S-358 to I-432; S-359 to I-432; S-360 to I-432; S-361 to I-432; T-362 to I-432; Y-363 to I-432; H-364 to I-432; A-365 to I-432; I-366 to I-432; I-367 to I-432; G-368 to I-432; G-369 to I-432; I-370 to I-432; V-371 to I-432; A-372 to I-432; F-373 to I-432; I-374 to I-432; V-375 to I-432; F-376 to I-432; L-377 to I-432; L-378 to I-432; L-379 to I-432; I-380 to I-432; M-381 to I-432; L-382 to I-432; I-383 to I-432; F-384 to I-432; L-385 to I-432; G-386 to I-432; H-387 to I-432; Y-388 to I-432; L-389 to I-432; I-390 to I-432; R-391 to I-432; H-392 to I-432; K-393 to I-432; G-394 to I-432; T-395 to I-432; Y-396 to I-432; L-397 to I-432; T-398 to I-432; H-399 to I-432; E-400 to I-432; A-401 to I-432; K-402 to I-432; G-403 to I-432; S-404 to I-432; D-405 to I-432; D-406 to I-432; A-407 to I-432; P-408 to I-432; D-409 to I-432; A-410 to I-432; D-411 to I-432; T-412 to I-432; A-413 to I-432; I-414 to I-432; I-415 to I-432; N-416 to I-432; A-417 to I-432; E-418 to I-432; G-419 to I-432; G-420 to I-432; Q-421 to I-432; S-422 to I-432; G-423 to I-432; G-424 to I-432; D-425 to I-432; D-426 to I-432; and/or K-427 to I-432 of SEQ ID NO: 361. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the following group of C-terminal deletions: M-1 to F-431; M-1 to Y-430; M-1 to E-429; M-1 to K-428; M-1 to K-427; M-1 to D-426; M-1 to D-425; M-1 to G-424; M-1 to G-423; M-1 to S-422; M-1 to Q-421; M-1 to G-420; M-1 to G-419; M-1 to E-418; M-1 to A-417; M-1 to N-416; M-1 to I-415; M-1 to I-414; M-1 to A-413; M-1 to T-412; M-1 to D-411; M-1 to A-410; M-1 to D-409; M-1 to P-408; M-1 to A-407; M-1 to D-406; M-1 to D-405; M-1 to S-404; M-1 to G-403; M-1 to K-402; M-1 to A-401; M-1 to E-400; M-1 to H-399; M-1 to T-398; M-1 to L-397; M-1 to Y-396; M-1 to T-395; M-1 to G-394; M-1 to K-393; M-1 to H-392; M-1 to R-391; M-1 to I-390; M-1 to L-389; M-1 to Y-388; M-1 to H-387; M-1 to G-386; M-1 to L-385; M-1 to F-384; M-1 to I-383; M-1 to L-382; M-1 to M-381; M-1 to I-380; M-1 to L-379; M-1 to L-378; M-1 to L-377; M-1 to F-376; M-1 to V-375; M-1 to I-374; M-1 to F-373; M-1 to A-

372; M-1 to V-371; M-1 to I-370; M-1 to G-369; M-1 to G-368; M-1 to I-367; M-1 to I-366; M-1 to A-365; M-1 to H-364; M-1 to Y-363; M-1 to T-362; M-1 to S-361; M-1 to S-360; M-1 to S-359; M-1 to S-358; M-1 to P-357; M-1 to V-356; M-1 to P-355; M-1 to S-354; M-1 to P-353; M-1 to D-352; M-1 to N-351; M-1 to V-350; M-1 to N-349; M-1 to L-348; M-1 to T-347; M-1 to Y-346; M-1 to Y-345; M-1 to A-344; M-1 to K-343; M-1 to Y-342; M-1 to S-341; M-1 to G-340; M-1 to M-339; M-1 to N-338; M-1 to S-337; M-1 to T-336; M-1 to A-335; M-1 to T-334; M-1 to C-333; M-1 to G-332; M-1 to Y-331; M-1 to T-330; M-1 to G-329; M-1 to S-328; M-1 to D-327; M-1 to S-326; M-1 to K-325; M-1 to N-324; M-1 to L-323; M-1 to F-322; M-1 to P-321; M-1 to F-320; M-1 to I-319; M-1 to L-318; M-1 to A-317; M-1 to S-316; M-1 to E-315; M-1 to Q-314; M-1 to T-313; M-1 to M-312; M-1 to K-311; M-1 to L-310; M-1 to P-309; M-1 to P-308; M-1 to V-307; M-1 to S-306; M-1 to G-305; M-1 to E-304; M-1 to K-303; M-1 to E-302; M-1 to W-301; M-1 to L-300; M-1 to Y-299; M-1 to Q-298; M-1 to Q-297; M-1 to P-296; M-1 to V-295; M-1 to P-294; M-1 to N-293; M-1 to G-292; M-1 to R-291; M-1 to G-290; M-1 to E-289; M-1 to C-288; M-1 to H-287; M-1 to L-286; M-1 to L-285; M-1 to L-284; M-1 to K-283; M-1 to Q-282; M-1 to G-281; M-1 to E-280; M-1 to R-279; M-1 to P-278; M-1 to H-277; M-1 to P-276; M-1 to P-275; M-1 to D-274; M-1 to P-273; M-1 to R-272; M-1 to I-271; M-1 to M-270; M-1 to A-269; M-1 to T-268; M-1 to P-267; M-1 to T-266; M-1 to Y-265; M-1 to L-264; M-1 to V-263; M-1 to E-262; M-1 to I-261; M-1 to R-260; M-1 to Q-259; M-1 to S-258; M-1 to T-257; M-1 to S-256; M-1 to R-255; M-1 to D-254; M-1 to A-253; M-1 to G-252; M-1 to K-251; M-1 to L-250; M-1 to S-249; M-1 to E-248; M-1 to H-247; M-1 to N-246; M-1 to V-245; M-1 to S-244; M-1 to C-243; M-1 to V-242; M-1 to I-241; M-1 to S-240; M-1 to A-239; M-1 to G-238; M-1 to D-237; M-1 to D-236; M-1 to E-235; M-1 to R-234; M-1 to T-233; M-1 to V-232; M-1 to Q-231; M-1 to F-230; M-1 to T-229; M-1 to V-228; M-1 to S-227; M-1 to S-226; M-1 to S-225; M-1 to V-224; M-1 to T-223; M-1 to F-222; M-1 to T-221; M-1 to K-220; M-1 to G-219; M-1 to N-218; M-1 to P-217; M-1 to D-216; M-1 to E-215; M-1 to Q-214; M-1 to I-213; M-1 to R-212; M-1 to T-211; M-1 to P-210; M-1 to E-209; M-1 to G-208; M-1 to H-207; M-1 to L-206; M-1 to E-205; M-1 to Q-204; M-1 to D-203; M-1 to G-202; M-1 to K-201; M-1 to R-200; M-1 to W-199; M-1 to T-198; M-1 to L-197; M-1 to R-196; M-1 to A-195; M-1 to A-194; M-1 to P-193; M-1 to K-192; M-1 to S-191; M-1

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to G-190; M-1 to S-189; M-1 to S-188; M-1 to Q-187; M-1 to C-186; M-1 to N-185; M-1 to L-184; M-1 to T-183; M-1 to A-182; M-1 to T-181; M-1 to D-180; M-1 to K-179; M-1 to E-178; M-1 to R-177; M-1 to L-176; M-1 to S-175; M-1 to S-174; M-1 to K-173; M-1 to Y-172; M-1 to G-171; M-1 to T-170; M-1 to I-169; M-1 to I-168;

5 M-1 to P-167; M-1 to K-166; M-1 to Q-165; M-1 to P-164; M-1 to I-163; M-1 to G-162; M-1 to L-161; M-1 to V-160; M-1 to T-159; M-1 to V-158; M-1 to L-157; M-1 to S-156; M-1 to K-155; M-1 to A-154; M-1 to T-153; M-1 to R-152; M-1 to V-151; M-1 to P-150; M-1 to M-149; M-1 to T-148; M-1 to F-147; M-1 to I-146; M-1 to S-145; M-1 to C-144; M-1 to T-143; M-1 to Y-142; M-1 to E-141; M-1 to G-140; M-1

10 to E-139; M-1 to D-138; M-1 to A-137; M-1 to L-136; M-1 to A-135; M-1 to V-134; M-1 to N-133; M-1 to S-132; M-1 to I-131; M-1 to S-130; M-1 to I-129; M-1 to S-128; M-1 to L-127; M-1 to E-126; M-1 to H-125; M-1 to P-124; M-1 to T-123; M-1 to S-122; M-1 to T-121; M-1 to V-120; M-1 to L-119; M-1 to Q-118; M-1 to I-117; M-1 to R-116; M-1 to N-115; M-1 to D-114; M-1 to R-113; M-1 to L-112; M-1 to A-

15 111; M-1 to R-110; M-1 to K-109; M-1 to E-108; M-1 to G-107; M-1 to F-106; M-1 to Y-105; M-1 to L-104; M-1 to T-103; M-1 to Q-102; M-1 to Q-101; M-1 to A-100; M-1 to P-99; M-1 to N-98; M-1 to S-97; M-1 to W-96; M-1 to Q-95; M-1 to L-94; M-1 to S-93; M-1 to S-92; M-1 to D-91; M-1 to E-90; M-1 to H-89; M-1 to D-88; M-1 to K-87; M-1 to V-86; M-1 to Q-85; M-1 to C-84; M-1 to K-83; M-1 to L-82; M-1 to

20 V-81; M-1 to V-80; M-1 to T-79; M-1 to G-78; M-1 to G-77; M-1 to A-76; M-1 to V-75; M-1 to V-74; M-1 to T-73; M-1 to E-72; M-1 to D-71; M-1 to S-70; M-1 to T-69; M-1 to W-68; M-1 to P-67; M-1 to Q-66; M-1 to S-65; M-1 to D-64; M-1 to Q-63; M-1 to S-62; M-1 to A-61; M-1 to L-60; M-1 to M-59; M-1 to D-58; M-1 to P-57; M-1 to S-56; M-1 to S-55; M-1 to W-54; M-1 to V-53; M-1 to T-52; M-1 to S-51; M-1

25 to S-50; M-1 to I-49; M-1 to A-48; M-1 to E-47; M-1 to D-46; M-1 to L-45; M-1 to P-44; M-1 to A-43; M-1 to L-42; M-1 to T-41; M-1 to G-40; M-1 to L-39; M-1 to E-38; M-1 to L-37; M-1 to D-36; M-1 to Q-35; M-1 to E-34; M-1 to Q-33; M-1 to W-32; M-1 to Y-31; M-1 to G-30; M-1 to D-29; M-1 to Q-28; M-1 to S-27; M-1 to L-26; M-1 to N-25; M-1 to A-24; M-1 to G-23; M-1 to G-22; M-1 to P-21; M-1 to A-20; M-

30 1 to W-19; M-1 to C-18; M-1 to C-17; M-1 to A-16; M-1 to F-15; M-1 to L-14; M-1 to L-13; M-1 to L-12; M-1 to L-11; M-1 to L-10; M-1 to L-9; M-1 to L-8; and/or M-1

to S-7 of SEQ ID NO: 361. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein (e.g., ability to inhibit the Mixed Lymphocyte Reaction), other functional activities (e.g., biological activities, ability to multimerize, ability to bind ligand, ability to generate antibodies, ability to bind antibodies) may still be retained. For example, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response. Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxyl terminus of the amino acid sequence of the polypeptide shown in Figures 3A-3C (SEQ ID NO: 361), as described by the general formula 1-n, where n is an integer from 6 to 432, where n corresponds to the position of the amino acid residue identified in SEQ ID NO: 361.

More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group of N-terminal deletions of the mature extracellular portion of the B7-H4 protein (SEQ ID NO: 1238): L-26 to G-368; S-27 to G-368; Q-28 to G-368; D-29 to G-368; G-30 to G-368; Y-31 to G-368; W-32 to G-368; Q-33 to G-368; E-34 to G-368; Q-35 to G-368; D-36 to G-368; L-37 to G-368; E-38 to G-368; L-39 to G-368; G-40 to G-368; T-41 to G-368; L-42 to G-368; A-43 to G-368; P-44 to G-368; L-45 to G-368; D-46 to G-368; E-47 to G-368; A-48 to G-368; I-49 to G-368; S-50 to G-368; S-51 to G-368; T-52 to G-368; V-53 to G-368; W-54 to G-368; S-55 to G-368; S-56 to G-368; P-57 to G-368; D-58 to G-368; M-59 to G-368; L-60 to G-

368; A-61 to G-368; S-62 to G-368; Q-63 to G-368; D-64 to G-368; S-65 to G-368;  
 Q-66 to G-368; P-67 to G-368; W-68 to G-368; T-69 to G-368; S-70 to G-368; D-71  
 to G-368; E-72 to G-368; T-73 to G-368; V-74 to G-368; V-75 to G-368; A-76 to G-  
 368; G-77 to G-368; G-78 to G-368; T-79 to G-368; V-80 to G-368; V-81 to G-368;  
 5 L-82 to G-368; K-83 to G-368; C-84 to G-368; Q-85 to G-368; V-86 to G-368; K-87  
 to G-368; D-88 to G-368; H-89 to G-368; E-90 to G-368; D-91 to G-368; S-92 to G-  
 368; S-93 to G-368; L-94 to G-368; Q-95 to G-368; W-96 to G-368; S-97 to G-368;  
 N-98 to G-368; P-99 to G-368; A-100 to G-368; Q-101 to G-368; Q-102 to G-368; T-  
 103 to G-368; L-104 to G-368; Y-105 to G-368; F-106 to G-368; G-107 to G-368; E-  
 10 108 to G-368; K-109 to G-368; R-110 to G-368; A-111 to G-368; L-112 to G-368; R-  
 113 to G-368; D-114 to G-368; N-115 to G-368; R-116 to G-368; I-117 to G-368; Q-  
 118 to G-368; L-119 to G-368; V-120 to G-368; T-121 to G-368; S-122 to G-368; T-  
 123 to G-368; P-124 to G-368; H-125 to G-368; E-126 to G-368; L-127 to G-368; S-  
 128 to G-368; I-129 to G-368; S-130 to G-368; I-131 to G-368; S-132 to G-368; N-  
 15 133 to G-368; V-134 to G-368; A-135 to G-368; L-136 to G-368; A-137 to G-368; D-  
 138 to G-368; E-139 to G-368; G-140 to G-368; E-141 to G-368; Y-142 to G-368; T-  
 143 to G-368; C-144 to G-368; S-145 to G-368; I-146 to G-368; F-147 to G-368; T-  
 148 to G-368; M-149 to G-368; P-150 to G-368; V-151 to G-368; R-152 to G-368; T-  
 153 to G-368; A-154 to G-368; K-155 to G-368; S-156 to G-368; L-157 to G-368; V-  
 20 158 to G-368; T-159 to G-368; V-160 to G-368; L-161 to G-368; G-162 to G-368; I-  
 163 to G-368; P-164 to G-368; Q-165 to G-368; K-166 to G-368; P-167 to G-368; I-  
 168 to G-368; I-169 to G-368; T-170 to G-368; G-171 to G-368; Y-172 to G-368; K-  
 173 to G-368; S-174 to G-368; S-175 to G-368; L-176 to G-368; R-177 to G-368; E-  
 178 to G-368; K-179 to G-368; D-180 to G-368; T-181 to G-368; A-182 to G-368; T-  
 25 183 to G-368; L-184 to G-368; N-185 to G-368; C-186 to G-368; Q-187 to G-368; S-  
 188 to G-368; S-189 to G-368; G-190 to G-368; S-191 to G-368; K-192 to G-368; P-  
 193 to G-368; A-194 to G-368; A-195 to G-368; R-196 to G-368; L-197 to G-368; T-  
 198 to G-368; W-199 to G-368; R-200 to G-368; K-201 to G-368; G-202 to G-368;  
 D-203 to G-368; Q-204 to G-368; E-205 to G-368; L-206 to G-368; H-207 to G-368;  
 30 G-208 to G-368; E-209 to G-368; P-210 to G-368; T-211 to G-368; R-212 to G-368;  
 I-213 to G-368; Q-214 to G-368; E-215 to G-368; D-216 to G-368; P-217 to G-368;  
 N-218 to G-368; G-219 to G-368; K-220 to G-368; T-221 to G-368; F-222 to G-368;

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T-223 to G-368; V-224 to G-368; S-225 to G-368; S-226 to G-368; S-227 to G-368;  
 V-228 to G-368; T-229 to G-368; F-230 to G-368; Q-231 to G-368; V-232 to G-368;  
 T-233 to G-368; R-234 to G-368; E-235 to G-368; D-236 to G-368; D-237 to G-368;  
 G-238 to G-368; A-239 to G-368; S-240 to G-368; I-241 to G-368; V-242 to G-368;  
 5 C-243 to G-368; S-244 to G-368; V-245 to G-368; N-246 to G-368; H-247 to G-368;  
 E-248 to G-368; S-249 to G-368; L-250 to G-368; K-251 to G-368; G-252 to G-368;  
 A-253 to G-368; D-254 to G-368; R-255 to G-368; S-256 to G-368; T-257 to G-368;  
 S-258 to G-368; Q-259 to G-368; R-260 to G-368; I-261 to G-368; E-262 to G-368;  
 V-263 to G-368; L-264 to G-368; Y-265 to G-368; T-266 to G-368; P-267 to G-368;  
 10 T-268 to G-368; A-269 to G-368; M-270 to G-368; I-271 to G-368; R-272 to G-368;  
 P-273 to G-368; D-274 to G-368; P-275 to G-368; P-276 to G-368; H-277 to G-368;  
 P-278 to G-368; R-279 to G-368; E-280 to G-368; G-281 to G-368; Q-282 to G-368;  
 K-283 to G-368; L-284 to G-368; L-285 to G-368; L-286 to G-368; H-287 to G-368;  
 C-288 to G-368; E-289 to G-368; G-290 to G-368; R-291 to G-368; G-292 to G-368;  
 15 N-293 to G-368; P-294 to G-368; V-295 to G-368; P-296 to G-368; Q-297 to G-368;  
 Q-298 to G-368; Y-299 to G-368; L-300 to G-368; W-301 to G-368; E-302 to G-368;  
 K-303 to G-368; E-304 to G-368; G-305 to G-368; S-306 to G-368; V-307 to G-368;  
 P-308 to G-368; P-309 to G-368; L-310 to G-368; K-311 to G-368; M-312 to G-368;  
 T-313 to G-368; Q-314 to G-368; E-315 to G-368; S-316 to G-368; A-317 to G-368;  
 20 L-318 to G-368; I-319 to G-368; F-320 to G-368; P-321 to G-368; F-322 to G-368; L-  
 323 to G-368; N-324 to G-368; K-325 to G-368; S-326 to G-368; D-327 to G-368; S-  
 328 to G-368; G-329 to G-368; T-330 to G-368; Y-331 to G-368; G-332 to G-368; C-  
 333 to G-368; T-334 to G-368; A-335 to G-368; T-336 to G-368; S-337 to G-368; N-  
 338 to G-368; M-339 to G-368; G-340 to G-368; S-341 to G-368; Y-342 to G-368;  
 25 K-343 to G-368; A-344 to G-368; Y-345 to G-368; Y-346 to G-368; T-347 to G-368;  
 L-348 to G-368; N-349 to G-368; V-350 to G-368; N-351 to G-368; D-352 to G-368;  
 P-353 to G-368; S-354 to G-368; P-355 to G-368; V-356 to G-368; P-357 to G-368;  
 S-358 to G-368; S-359 to G-368; S-360 to G-368; S-361 to G-368; T-362 to G-368;  
 and/or Y-363 to G-368 of SEQ ID NO: 1238. Polypeptides encoded by these  
 30 polynucleotides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides  
 comprising, or alternatively consisting of, an amino acid sequence selected from the

group of C-terminal deletions of the mature extracellular portion of the B7-H4 protein (SEQ ID NO: 1238): N-25 to I-367; N-25 to I-366; N-25 to A-365; N-25 to H-364; N-25 to Y-363; N-25 to T-362; N-25 to S-361; N-25 to S-360; N-25 to S-359; N-25 to S-358; N-25 to P-357; N-25 to V-356; N-25 to P-355; N-25 to S-354; N-25 to P-353;

5 N-25 to D-352; N-25 to N-351; N-25 to V-350; N-25 to N-349; N-25 to L-348; N-25 to T-347; N-25 to Y-346; N-25 to Y-345; N-25 to A-344; N-25 to K-343; N-25 to Y-342; N-25 to S-341; N-25 to G-340; N-25 to M-339; N-25 to N-338; N-25 to S-337; N-25 to T-336; N-25 to A-335; N-25 to T-334; N-25 to C-333; N-25 to G-332; N-25 to Y-331; N-25 to T-330; N-25 to G-329; N-25 to S-328; N-25 to D-327; N-25 to S-

10 326; N-25 to K-325; N-25 to N-324; N-25 to L-323; N-25 to F-322; N-25 to P-321; N-25 to F-320; N-25 to I-319; N-25 to L-318; N-25 to A-317; N-25 to S-316; N-25 to E-315; N-25 to Q-314; N-25 to T-313; N-25 to M-312; N-25 to K-311; N-25 to L-310; N-25 to P-309; N-25 to P-308; N-25 to V-307; N-25 to S-306; N-25 to G-305; N-25 to E-304; N-25 to K-303; N-25 to E-302; N-25 to W-301; N-25 to L-300; N-25

15 to Y-299; N-25 to Q-298; N-25 to Q-297; N-25 to P-296; N-25 to V-295; N-25 to P-294; N-25 to N-293; N-25 to G-292; N-25 to R-291; N-25 to G-290; N-25 to E-289; N-25 to C-288; N-25 to H-287; N-25 to L-286; N-25 to L-285; N-25 to L-284; N-25 to K-283; N-25 to Q-282; N-25 to G-281; N-25 to E-280; N-25 to R-279; N-25 to P-278; N-25 to H-277; N-25 to P-276; N-25 to P-275; N-25 to D-274; N-25 to P-273;

20 N-25 to R-272; N-25 to I-271; N-25 to M-270; N-25 to A-269; N-25 to T-268; N-25 to P-267; N-25 to T-266; N-25 to Y-265; N-25 to L-264; N-25 to V-263; N-25 to E-262; N-25 to I-261; N-25 to R-260; N-25 to Q-259; N-25 to S-258; N-25 to T-257; N-25 to S-256; N-25 to R-255; N-25 to D-254; N-25 to A-253; N-25 to G-252; N-25 to K-251; N-25 to L-250; N-25 to S-249; N-25 to E-248; N-25 to H-247; N-25 to N-246;

25 N-25 to V-245; N-25 to S-244; N-25 to C-243; N-25 to V-242; N-25 to I-241; N-25 to S-240; N-25 to A-239; N-25 to G-238; N-25 to D-237; N-25 to D-236; N-25 to E-235; N-25 to R-234; N-25 to T-233; N-25 to V-232; N-25 to Q-231; N-25 to F-230; N-25 to T-229; N-25 to V-228; N-25 to S-227; N-25 to S-226; N-25 to S-225; N-25 to V-224; N-25 to T-223; N-25 to F-222; N-25 to T-221; N-25 to K-220; N-25 to G-219;

30 N-25 to N-218; N-25 to P-217; N-25 to D-216; N-25 to E-215; N-25 to Q-214; N-25 to I-213; N-25 to R-212; N-25 to T-211; N-25 to P-210; N-25 to E-209; N-25 to G-208; N-25 to H-207; N-25 to L-206; N-25 to E-205; N-25 to Q-204; N-25 to D-203;

N-25 to G-202; N-25 to K-201; N-25 to R-200; N-25 to W-199; N-25 to T-198; N-25 to L-197; N-25 to R-196; N-25 to A-195; N-25 to A-194; N-25 to P-193; N-25 to K-192; N-25 to S-191; N-25 to G-190; N-25 to S-189; N-25 to S-188; N-25 to Q-187; N-25 to C-186; N-25 to N-185; N-25 to L-184; N-25 to T-183; N-25 to A-182; N-25 to T-181; N-25 to D-180; N-25 to K-179; N-25 to E-178; N-25 to R-177; N-25 to L-176; N-25 to S-175; N-25 to S-174; N-25 to K-173; N-25 to Y-172; N-25 to G-171; N-25 to T-170; N-25 to I-169; N-25 to I-168; N-25 to P-167; N-25 to K-166; N-25 to Q-165; N-25 to P-164; N-25 to I-163; N-25 to G-162; N-25 to L-161; N-25 to V-160; N-25 to T-159; N-25 to V-158; N-25 to L-157; N-25 to S-156; N-25 to K-155; N-25 to A-154; N-25 to T-153; N-25 to R-152; N-25 to V-151; N-25 to P-150; N-25 to M-149; N-25 to T-148; N-25 to F-147; N-25 to I-146; N-25 to S-145; N-25 to C-144; N-25 to T-143; N-25 to Y-142; N-25 to E-141; N-25 to G-140; N-25 to E-139; N-25 to D-138; N-25 to A-137; N-25 to L-136; N-25 to A-135; N-25 to V-134; N-25 to N-133; N-25 to S-132; N-25 to I-131; N-25 to S-130; N-25 to I-129; N-25 to S-128; N-25 to L-127; N-25 to E-126; N-25 to H-125; N-25 to P-124; N-25 to T-123; N-25 to S-122; N-25 to T-121; N-25 to V-120; N-25 to L-119; N-25 to Q-118; N-25 to I-117; N-25 to R-116; N-25 to N-115; N-25 to D-114; N-25 to R-113; N-25 to L-112; N-25 to A-111; N-25 to R-110; N-25 to K-109; N-25 to E-108; N-25 to G-107; N-25 to F-106; N-25 to Y-105; N-25 to L-104; N-25 to T-103; N-25 to Q-102; N-25 to Q-101; N-25 to A-100; N-25 to P-99; N-25 to N-98; N-25 to S-97; N-25 to W-96; N-25 to Q-95; N-25 to L-94; N-25 to S-93; N-25 to S-92; N-25 to D-91; N-25 to E-90; N-25 to H-89; N-25 to D-88; N-25 to K-87; N-25 to V-86; N-25 to Q-85; N-25 to C-84; N-25 to K-83; N-25 to L-82; N-25 to V-81; N-25 to V-80; N-25 to T-79; N-25 to G-78; N-25 to G-77; N-25 to A-76; N-25 to V-75; N-25 to V-74; N-25 to T-73; N-25 to E-72; N-25 to D-71; N-25 to S-70; N-25 to T-69; N-25 to W-68; N-25 to P-67; N-25 to Q-66; N-25 to S-65; N-25 to D-64; N-25 to Q-63; N-25 to S-62; N-25 to A-61; N-25 to L-60; N-25 to M-59; N-25 to D-58; N-25 to P-57; N-25 to S-56; N-25 to S-55; N-25 to W-54; N-25 to V-53; N-25 to T-52; N-25 to S-51; N-25 to S-50; N-25 to I-49; N-25 to A-48; N-25 to E-47; N-25 to D-46; N-25 to L-45; N-25 to P-44; N-25 to A-43; N-25 to L-42; N-25 to T-41; N-25 to G-40; N-25 to L-39; N-25 to E-38; N-25 to L-37; N-25 to D-36; N-25 to Q-35; N-25 to E-34; N-25 to Q-33; N-25 to W-32; and/or

N-25 to Y-31 of SEQ ID NO: 1238. Polypeptides encoded by these polynucleotides are also encompassed by the invention.

In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides comprising, or alternatively consisting of, one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO: 361, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present invention is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set forth herein as m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also included are polynucleotide sequences encoding a polypeptide consisting of a portion of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. 209007 (deposited on April 28, 1997) and 209083 (deposited on May 29, 1997), where this portion excludes any integer of amino acid residues from 1 to about 228 amino acids from the amino terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC Deposit Nos. 209007 and 209083, or any integer of amino acid residues from 1 to about 228 amino acids from the carboxyl terminus, or any combination of the above amino terminal and carboxyl terminal deletions, of the complete amino acid sequence encoded by the cDNA clone contained in ATCC Deposit Nos. 209007 and 209083. Polypeptides encoded by these polynucleotides also are encompassed by the invention.

As described herein or otherwise known in the art, the polynucleotides of the invention have uses that include, but are not limited to, serving as probes or primers in chromosome identification, chromosome mapping, and linkage analysis.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of CNS and/or immune system tissue(s) or cell type(s)

present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases and/or disorders involving immune system activation, stimulation and/or surveillance, particularly involving T cells and/or neutrophils, susceptibility to viral disease and diseases of the CNS, especially cancers of that system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). Particularly contemplated are the use of antibodies directed against the extracellular portion of this protein which act as antagonists and/or agonists for the activity of the B7-H4 protein. Such antagonistic/agonist antibodies would be useful for the prevention and/or inhibition of such biological activities as are disclosed herein (e.g., T cell modulated activities).

For a number of disorders of the above tissues or cells, particularly of the immune system and CNS, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, CNS, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The homology to members of the B7 family of ligands indicates that the polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, detection and/or treatment of diseases and/or disorders involving immune system activation, stimulation and/or surveillance, particularly as relating to T cells and/or neutrophils. In particular, the translation product of the B7-H4 gene may be involved in the costimulation of T cells, binding to ICOS, and/or may play a role in modulation of the expression of particular cytokines.

More generally, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g., by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as

an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

The tissue distribution and homology to poliovirus receptor precursors suggests that the protein product of this clone would be useful for the treatment and prevention of diseases that involve the binding and uptake of virus particles for infection. It might also be helpful in genetic therapy where the goal is to insert foreign DNA into infected cells. With the help of this protein, the binding and uptake of this foreign DNA might be aided. In addition, it is expected that over expression of this gene will indicate abnormalities involving the CNS, particularly cancers of that system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 123 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2523 of SEQ ID NO: 123, b is an integer of 15 to 2537, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO: 123

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 114**

The translation product of this gene shares sequence homology with YO87\_CAEEL hypothetical 28.5 KD protein ZK1236.7 in chromosome III of *Caenorhabditis elegans* in addition to alpha-1 collagen type III (See Genbank Accession No. gi|537432). In specific embodiments, polypeptides of the invention comprise, or alternatively

5 consists of, an amino acid sequence selected from the group:

VPCLPDRVHQLHQA VQGICALGRPGFPGGP THSGHHKSHPGPAGGDYNRCDR  
PGQVHLHNPRGTGRRGQLHPTAGPGVHRRACPSQQLPHRLGPGVPCPSPSLT  
PVLPSWTQSWCGLPGYTSSS (SEQ ID NO:954),

VHQLHQA VQGICALGRPGFPGGP (SEQ ID NO:955),

10 PTHSGHHKSHPGPAGGDYNRCDRPGQVHLHNPRGTGRRGQLH (SEQ ID  
NO:956), and/or

LHPTAGPGVHRRACPSQQLPHRLGPGVPCPSPSLTPVLPSWTQSWCGLPGYTS  
SS (SEQ ID NO:957). Moreover, fragments and variants of these polypeptides (such

as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
15 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides ) are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding these polypeptides are also encompassed by the  
20 invention.

This gene is expressed primarily in brain cells, and to a lesser extent in  
activated B and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
25 and for diagnosis of diseases and conditions which include, but are not limited to,  
neurodegeneration and immunological disorders. Similarly, polypeptides and  
antibodies directed to these polypeptides are useful in providing immunological  
probes for differential identification of the tissue(s) or cell type(s). For a number of  
disorders of the above tissues or cells, particularly of the neural and immune systems,  
30 expression of this gene at significantly higher or lower levels may be routinely  
detected in certain tissues or cell types (e.g. brain, immune, cancerous and wounded  
tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal

fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID

- 5 NO: 362 as residues: Glu-34 to Glu-39, Gly-51 to Ser-72, Ala-88 to Glu-93, Gln-100 to Val-105.

- The tissue distribution in brain cells, combined with the homology to YO87\_CAEEL hypothetical 28.5 KD protein ZK1236.7 in chromosome III of *Caenorhabditis elegans* as well as to a conserved alpha-1 collagen type III protein indicates that the protein product of this gene is useful for the detection and treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorders. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:124 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1376 of SEQ ID NO:124, b is an integer of 15 to 1390, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:124, and where b is greater than or equal to a + 14.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 115**



The translation product of this gene shares sequence homology with alpha 3 type IX collagen, which is thought to be important in hyaline cartilage formation via its ability to uptake inorganic sulfate by cells (See Genbank Accession No. gi|975657).

5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
SLRRPRSAAXQTLTTLSSVSSASSSALPGSREPCDPRAPPPRSGSAASCCSCC  
CSCPRRRAPLRSRPGSKRRIRQREVVDLYNGMCLQGPAGVPGRDGSFGANGI  
PGTPGIPGRDGFKGKGECLRESFEESWTPNYKQCSWSSLNYGIDLGKIAECT  
10 FTKMRSNSALRVLFSGSLRLKCRNACCQRWYFTFNGAECSGPLPIEAIYLDQ  
GSPEMNSTINIHR TSSVEGLCEGIGAGLVDVAIWVGTCSDYPKGDASTGWNS  
VSRIII EELPK (SEQ ID NO:958),  
SLRRPRSAAXQTLTTLSSVSSASSSALPGSREPCDPRAPPPRSGSAASCCSCC  
CSCPRR (SEQ ID NO:959),  
15 RAPLRSRPGSKRRIRQREVVDLYNGMCLQGPAGVPGRDGSFGANGIPGTPGI  
(SEQ ID NO:960),  
TPGIPGRDGFKGKGECLRESFEESWTPNYKQCSWSSLNYGIDLGKIAECTF  
(SEQ ID NO:961),  
FTKMRSNSALRVLFSGSLRLKCRNACCQRWYFTFNGAECSGPLPIEAIYLDQ  
20 GSPEMNSTINIHR (SEQ ID NO:962), and/or  
RTSSVEGLCEGIGAGLVDVAIWVGTCSDYPKGDASTGWNSVSRIII EELPK  
(SEQ ID NO:963). Moreover, fragments and variants of these polypeptides (such as,  
for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
25 encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides ) are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

30 This gene is expressed primarily in smooth muscle, and to a lesser extent in  
synovial tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias, i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid and autoimmune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. muscle, synovial tissues, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in smooth muscle, and homology to alpha 3 type IX collagen indicates that the protein product of this gene is useful for the treatment and diagnosis of diseases associated with the mutation in this gene which leads to the many different types of chondrodysplasias. By the use of this product, the abnormal growth and development of bones of the limbs and spine could be detected or treated *in utero*, since the protein or polypeptides thereof could affect epithelial cells early in development, and later the chondrocytes of the developing craniofacial structure. In addition, the expression of this gene product in synovium would suggest a role in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis as well as disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Moreover, the expression within smooth muscle

indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, detection, and/or prevention of a variety of vascular disorders, which include, but are not limited to, atherosclerosis, embolism, stroke, aneurysm, or microvascular disease. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:125 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1274 of SEQ ID NO:125, b is an integer of 15 to 1288, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:125, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 116**

The translation product of this gene shares sequence homology with retrovirus-related reverse transcriptase, which is thought to be important in viral replication.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: TKKENC RPASLMNIDTKILNKILMNQ (SEQ ID NO:964). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are

also encompassed by the invention. (See Genbank Accession No. pir|A25313|GNHUL1).

This gene is expressed primarily in human meningioma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, retroviral diseases such as AIDS, and possibly certain cancers due to transactivation of latent cell division genes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. meningioma, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human meningioma, combined with the homology to a retrovirus-related reverse transcriptase indicates that the protein product of this gene is useful for the detection and treatment of diseases and conditions associated with retroviral infection, since a functional reverse transcriptase (RT) or RT-like molecule is an integral component of the retroviral life cycle. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:126 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1503 of SEQ ID NO:126, b is an

integer of 15 to 1517, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:126, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 117

The translation product of this gene shares sequence homology with an unknown gene from *C. elegans*, as well as weak homolog with mammalian metaxin, a gene contiguous to both thrombospondin 3 and glucocerebrosidase, and is known to be required for embryonic development. Recently another group cloned and sequenced this gene from humans, naming it metaxin 2. It is thought that metaxin 1 and metaxin 2 interact, and are associated with the mammalian mitochondrial outer membrane (See Genbank Accession No. AF053551).

In specific embodiments, polypeptides of the invention comprise, or alternatively

consists of, an amino acid sequence selected from the group:

MCNLPIKVVCRAEYMSPSGKVPXXHVGNGQVVSELGPIVQFVKAKGHSLSDGLEEVQKAEMKAYMELVNNMLLTAELYLQWCDEATVGXITHXRYGSPYPWPLXHILAYQKQWEVKRKXKAIGWGKKTLDQVLEDVDQCCQALSQRLGTQPYFFNKQPTELDALVFGHLYTILTTQLTNDELSEKVKNYSNLLAFCRRIEQHY

FED RGKGRLS (SEQ ID NO:965),

MCNLPIKVVCRAEYMSPSGKVPXXHVGNGQVVSELGPIVQFVK (SEQ ID

NO:966), FVKAKGHSLSDGLEEVQKAEMKAYMELVNNMLLTAELYLQWCDE

(SEQ ID NO:967), LQWCDEATVGXITHXRYGSPYPWP

LXHILAYQKQWEVKRKXKAIGWGKKTLD (SEQ ID NO:968),

DQVLEDVDQCCQ ALSQRLGTQPYFFNKQPTELDALVFGHLYTI (SEQ ID

NO:969), and/or LTTQLTNDELSEKVKNYSNLLAFCRRIEQHYPEDRGKGRLS

(SEQ ID NO:970). Moreover, fragments and variants of these polypeptides (such as,

for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,

95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides

encoded by the polynucleotide which hybridizes, under stringent conditions, to the

polynucleotide encoding these polypeptides ) are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the

invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. (See Genbank Accession No. gi|1326108).

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in  
5 linkage analysis for chromosome 2.

This gene is expressed primarily in fetal tissues, and to a lesser extent in hematopoietic cells and tissues, including spleen, monocytes, and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
10 and for diagnosis of diseases and conditions which include, but are not limited to, cancer; lymphoproliferative disorders; inflammation; chondrosarcoma, and Gaucher disease. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells,  
15 particularly of the hematopoietic and embryonic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, fetal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene  
20 expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal tissues indicates that the protein product of this gene is useful for the diagnosis and treatment of cancer and other proliferative disorders. Moreover, this protein may play a role in the regulation of cellular  
25 division. Additionally, the expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and survival of hematopoietic cell lineages. Thus, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor  
30 cells. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:127 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1059 of SEQ ID NO:127, b is an integer of 15 to 1073, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:127, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 118

The translation product of this gene shares sequence homology with reverse transcriptase, which is important in the synthesis of a cDNA chain from an RNA molecule, and is a method whereby the infecting RNA chains of retroviruses are transcribed into their DNA complements.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: MXXXNSHITIFTLNVNGLNAPNERHRLANWIQSQDVCCIQTETHLTGRDTHRLKIKGWRKIYQANGKQKK (SEQ ID NO:971), FTLNVNGLNAPNERHRLANWIQSQDQVC (SEQ ID NO:972), THLTGRDTHRLKIKGWR (SEQ ID NO:973), and/or GWRKIYQANGKQKK (SEQ ID NO:974). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. (See Genbank Accession No. gi|2072964).

This gene is expressed primarily in skin, and to a lesser extent in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancers; hematopoietic disorders; inflammation; disorders of immune surveillance. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the epidermis and/or hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. skin, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in skin, combined with the homology to a reverse transcriptase indicates that the protein product of this gene is useful for cancer therapy, particularly of the integumentary system. Expression in the skin also indicates that this gene is useful in wound healing and fibrosis. Expression by neutrophils also indicates that this gene product plays a role in inflammation and the control of immune surveillance (i.e., recognition of viral pathogens). Reverse transcriptase family members are also useful in the detection and treatment of AIDS. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:128 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 286 of SEQ ID NO:128, b is an



integer of 15 to 300, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:128, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 119

The translation product of this gene shares sequence homology with reverse transcriptase, which is important in the synthesis of a cDNA copy of an RNA molecule, and is a method whereby a retrovirus reverse-transcribes its genome into an inheritable DNA copy.

This gene is expressed primarily in the frontal cortex of brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and neurodegenerative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS and peripheral nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in the frontal cortex, combined with the homology to a reverse transcriptase suggest that this gene is useful in the treatment of cancer and AIDS, particularly of the neural system. The expression in brain indicates that it plays a role in neurodegenerative disorders and in neural degeneration. Furthermore, elevated expression of this gene product within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of

such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:129 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1261 of SEQ ID NO:129, b is an integer of 15 to 1275, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:129, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 120**

The translation product of this gene shares homology to a hypothetical protein in *Schizosaccharomyces pombe* (See Genbank Accession No. 2281980).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: IYHLHSWIFFHFKRAFCMCFITMKVIAHCSKLRKCXNAQIS VFCTTLTASYPT (SEQ ID NO:975), IYHLHSWIFFHFKRAFCMCFITM (SEQ ID NO:976), and/or KVIAHCSKLRKCXNAQISVFCTTLTASYPT (SEQ ID NO:977). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 18. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 18.

5 This gene is expressed primarily in adult hypothalamus and to a lesser extent in infant brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disorders; endocrine function; and vertigo. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, CNS and peripheral nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

15 The tissue distribution in adult hypothalamus and infant brain indicates that the protein product of this gene is useful for the treatment and diagnosis of neurodegenerative disorders; diagnosis of tumors of a brain or neuronal origin; treatments involving hormonal control of the entire body and of homeostasis, behavioral disorders, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:130 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 458 of SEQ ID NO:130, b is an integer of 15 to 472, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:130, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 121

The translation product of this gene shares sequence homology with the human IRLB protein which is thought to be important in binding to a c-myc promoter element and thus regulating its transcription (See Genbank Accession No. gi|33969). The gene encoding the disclosed cDNA is thought to reside on chromosome 1.

Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: WNLLWYFQRLRLPSILPGLVLASCDGPSXSQAPSPWLTPDPASVQVRLLWDV LTPDPN (SEQ ID NO:978), QRGYREILFLTMAALGKDHVDIVAFDKKYKSAF NKLASSMGKEELRHRAQMP (SEQ ID NO:979), and/or WNLLWYFQRLRLP SILPGLVLAS (SEQ ID NO:980). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in brain and breast, and to a lesser extent in a variety of hematopoietic tissues and cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer of the brain and breast; lymphoproliferative disorders; neurodegenerative diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the CNS, breast, and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, breast, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain indicates that the protein product of this gene is useful for the treatment and diagnosis of cancer of the brain, breast, and hematopoietic system. In addition, it is useful for the treatment of neurodegenerative disorders, as well as disorders of the hematopoietic system, including defects in immune competency and inflammation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:131 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1936 of SEQ ID NO:131, b is an integer of 15 to 1950, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:131, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 122

5 The translation product of this gene shares sequence homology with an ATP synthase, a key component of the proton channel that is thought to be important in the translocation of protons across the membrane.

This gene is expressed primarily in T-cell lymphoma.

10 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, T cell lymphoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or  
15 lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the  
20 disorder.

The tissue distribution in T-cell lymphoma, combined with the homology to an ATP synthase indicates that the protein product of this gene is useful for the treatment of defects in proton transport, homeostasis, and metabolism, as well as the diagnosis and treatment of lymphoma. Because the gene is expressed in cells of  
25 lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:132 and may have been publicly available prior to conception

of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 976 of SEQ ID NO:132, b is an integer of 15 to 990, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:132, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 123

The gene encoding the disclosed cDNA is thought to reside on chromosome 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

This gene is expressed primarily in a variety of fetal tissues, including fetal liver, lung, and spleen, and to a lesser extent in a variety of blood cells, including eosinophils and T cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer (abnormal cell proliferation); T cell lymphomas; and hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the fetus and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. fetal, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal tissues indicates that the protein product of this gene is useful for the treatment and diagnosis of conditions involving cell proliferation. Similarly, the fetal tissue expression, as well as the expression in a variety of blood cell lineages, indicates that it may play a role in either cellular proliferation, apoptosis, or cell survival. Thus it may be useful in the management and treatment of a variety of cancers and malignancies. In addition, its expression in blood cells indicates that it may play additional roles in hematopoietic disorders and conditions, and could be useful in treating diseases involving autoimmunity, immune modulation, immune surveillance, and inflammation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:133 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1706 of SEQ ID NO:133, b is an integer of 15 to 1720, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:133, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 124**

This gene is expressed primarily in placenta, and to a lesser extent in pineal gland and rhabdomyosarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental, endocrine, and female reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological



probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the placenta and endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. placental, endocrine, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 372 as residues: Leu-69 to Val-76.

The tissue distribution in placenta indicates that the protein product of this gene is useful for the diagnosis and treatment of developmental disorders. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:134 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

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cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 691 of SEQ ID NO:134, b is an integer of 15 to 705, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:134, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 125**

Contact of cells with supernatant expressing the product of this gene increases the permeability of THP-1 Monocyte cells to calcium. Thus, it is likely that the product of this gene is involved in a signal transduction pathway that is initiated when the product of this gene binds a receptor on the surface of the Monocyte cell. Thus, polynucleotides and polypeptides have uses which include, but are not limited to, activating monocyte cells.

This gene is expressed primarily in benign prostatic hyperplasia.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of benign prostatic hyperplasia. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in benign prostatic hyperplasia tissue indicates that the protein product of this gene is useful for the treatment and diagnosis of proliferative disorders of the prostate. Furthermore, the biological activity data indicates that the translation product of this gene is useful for the stimulation of certain immune system

cells, such as monocytes, which may be useful for helping the body to defend against infection. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:135 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 309 of SEQ ID NO:135, b is an integer of 15 to 323, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:135, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 126**

This gene is expressed primarily in Raji cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammation and T cell autoimmune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in Raji cells indicates that the protein product of this gene is useful for treatment and diagnosis of inflammation and T cell autoimmune disorders. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases (such as AIDS), and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:136 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 568 of SEQ ID NO:136, b is an integer of 15 to 582, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:136, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 127**

This gene is expressed primarily in apoptotic T-cells, and to a lesser extent in suppressor T cells and ulcerative colitis.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases involving premature apoptosis, and immunological and gastrointestinal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or

lower levels may be routinely detected in certain tissues or cell types (e.g. immune, gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 375 as residues: Asp-23 to Gly-29.

The tissue distribution in apoptotic T-cells indicates that the protein product of this gene is useful for the treatment and diagnosis of disorders involving inappropriate levels of apoptosis, especially in immune cell lineages. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases (such as AIDS), and leukemia. Furthermore, expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:137 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1007 of SEQ ID NO:137, b is an integer of 15 to 1021, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:137, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 128

The translation product of this gene shares sequence homology with an *C. elegans* coding region C47D12.2 of unknown function (See Genbank Accession No. gnl|PID|e348986).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

EDDGFNRSIHEVILKNITWYSERVLTEISLGSLILVVIRTIQYNMTRTRDKYLH  
 TNCLAALANMSAQFRSLHQYAAQRIISLFSLLSKKHNVLEQATQSLRGSLS  
 NDVPLPDYAQDLNVIEEVIRMMLEIINSCLTNSLHHNPNLVYALLYKRDLFEQ  
 FRTHPSFQDIMQNIDLVISFFSSRLLQAGS (SEQ ID NO:981),  
 10 EDDGFNRSIHEVILKNITWYSERVLTEISLGSLILVV (SEQ ID NO:982),  
 RTIQYNMTRTRDKYLHTNCLAALANMSAQFRSLHQYAAQRIISLFSLLSKKH  
 N (SEQ ID NO:983),  
 SCLTNSLHHNPNLVYALLYKRDLFEQFRTHPSFQDIMQNIDLVISFFSSRLLQA  
 GS (SEQ ID NO:984), KKHNVLEQATQSLRGSLSNDVPLPDYAQD (SEQ ID  
 15 NO:985), TISNSSFISGYNAKY (SEQ ID NO:986), and/or  
 LKVAASWELSCQWNGSWKSLSKASLRC PKTD (SEQ ID NO:987). Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 20 which hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome  
 25 18. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 18.

This gene is expressed primarily in smooth muscle, and to a lesser extent in  
 fetal liver/spleen.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 30 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 atherosclerosis and other cardiovascular and hepatic disorders. Similarly,

polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the circulatory system, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues or cell types (e.g. muscle, fetal liver/spleen, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the

10 disorder.

The tissue distribution in smooth muscle indicates that the protein product of this gene is useful for the diagnosis and treatment of circulatory system disorders such as atherosclerosis, hypertension, stroke, aneurysms, embolisms, and thrombosis. In addition, the tissue distribution indicates that the protein product of this gene is useful

15 for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition the expression in fetus indicates a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue

20 trauma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:138 and may have been publicly available prior to conception

25 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1763 of SEQ ID NO:138, b is an

30 integer of 15 to 1777, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:138, and where b is greater than or equal to a + 14.

# FEATURES OF PROTEIN ENCODED BY GENE NO: 129

The translation product of this gene shares sequence homology with a ribosomal protein which is thought to be important in cellular metabolism, in addition to the C.elegans protein F40F11.1 which does not have a known function at the current time (See Genbank Accession No. gnl|PID|e244552 ).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 10 MADIQTERAYQKQPTIFQNKKRVLLGETGKEKLPRVTNKNIGLGFKDTPRRL  
LRGTYIDKKCPFTGNVSIRGRILSGVVTQDEDAEDHCHPPRLSALHPQVQPLR  
EAPQEHVCTPVPLLQGRPDR (SEQ ID NO:988),  
MKMQRTIVIRRDYLHYIRKYNRFEKRRHKNMSVHLSPCFRDVQIGDIVTVGEC  
RPLSKTVRFNVLKVTKAAGTKKQFQKF (SEQ ID NO:989),
- 15 MADIQTERAYQKQPTIFQNKKRVLLGETGK (SEQ ID NO:990),  
KLPRVTNKNIGLGFKDTPRRLLRGTYIDKKCPFTGNVSIRGRILSGVVTQDED  
AEDHC (SEQ ID NO:991),  
HCHPPRLSALHPQVQPLREAPQEHVCTPVPLLQGRPDR (SEQ ID NO:992),  
MKMQRTIVIRRDYLHYIRKYNRFEKRRHKNMSVHLSP (SEQ ID NO:993),
- 20 CFRDVQIGDIVTVGECRPLSKTVRFNVLKVTKAAGTKKQFQKF (SEQ ID  
NO:994), PRLLRGTYIDKKCPFTGNVSIRGRILSGVVTQ (SEQ ID NO:995),  
SRGTGVQTCSCGASRGCTCGCSADSLGG (SEQ ID NO:996), and/or  
QWSSASSSWVTTPERIRPRMDTLPVKGHFLSM (SEQ ID NO:997). Moreover,  
25 fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
30 these polypeptides are also encompassed by the invention.



The gene encoding the disclosed cDNA is thought to reside on chromosome 19. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 19.

This gene is expressed primarily in Wilm's tumor, and to a lesser extent in thymus and stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, Kidney disorders and cancer, diseases affecting RNA translation. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the Wilm's tumors, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. kidney, thymus, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 377 as residues: Arg-15 to Gly-22.

The tissue distribution in Wilm's tumor, combined with the homology to a ribosomal protein indicates that the protein product of this gene is useful for diseases affecting RNA translation, in addition to proliferative disorders. Furthermore, given the tissue distribution, the translation product of this gene may be useful in treating and/or detecting Wilm's tumor or tumors of other tissues mentioned previously. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:139 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 629 of SEQ ID NO:139, b is an integer of 15 to 643, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:139, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 130

The translation product of this gene shares sequence homology with a yeast DNA helicase, which is thought to be important in global transcriptional regulation (See Genbank Accession No. gnl|PID|e243594).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

IFYDSDWNPTVDQQAMDRAHRLGQTKQVTYR LICKGTIEERILQRAKEKSEI  
QRMVISG (SEQ ID NO:998),  
TRMIDLLEEYMVYRKHTYXRLDGSSKISERRDMVADFQNRNDIFVLLSTRA  
GGLGINLTAXDTVHF (SEQ ID NO:999),  
IFYDSDWNPTVDQQAMDRAHRLGQTKQVTYR (SEQ ID NO:1000),  
VYRLICKGTIEERILQRAKEKSEIQRMVISG (SEQ ID NO:1001),  
TRMIDLLEEYMVYRKHTYXRLDGSSKISERRDM (SEQ ID NO:1002),  
RRDMVADFQNRNDIFVLLSTRAGGLGINLTAXDTVHF (SEQ ID NO:1003),  
IFYDSDWNPTVDQQAMDRAHRLGQTKQVTYR LICKG (SEQ ID NO:1004),  
IFYDSDWNPTVDQQAMDRAHRLGQTKQVTYR LICKG (SEQ ID NO:1005),  
RLICKGTIEERILQRAKEKSEIQRMVISG (SEQ ID NO:1006), and/or  
GTRMIDLLEEYMVYRKHTYXRLDGSSKISERRDMVADFQNRNDIFVLLSTR  
AGGLGINLTAXDTVHFL (SEQ ID NO:1007). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are

also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in amygdala.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, diseases and disorders of the brain and the endocrine system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, endocrine, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 378 as residues: Lys-24 to Tyr-34.

The tissue distribution in amygdala, combined with the homology to a DNA helicase indicates that the protein product of this gene is useful for diseases affecting RNA transcription, particularly developmental disorders and healing wounds, since the later are thought to approximate developmental transcriptional regulation. The amygdala processes sensory information and relays this to other areas of the brain including the endocrine and autonomic domains of the hypothalamus and the brain stem. Therefore, the translation product of this gene is also useful for the detection and/or treatment of disorders of the endocrine and/or neural systems. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:140 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1206 of SEQ ID NO:140, b is an integer of 15 to 1220, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:140, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 131

This gene is expressed primarily in prostate, and to a lesser extent in amygdala and pancreatic tumors.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate enlargement and gastrointestinal disorders, particularly of the pancreas and gall bladder. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in prostate indicates that the protein product of this gene is useful for the treatment and diagnosis of prostate or reproductive diseases, including benign prostatic hyperplasia and prostate cancer. In addition, the tissue distribution in tumors of the pancreas indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tissues where expression has been indicated. Protein, as well as, antibodies directed against

the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:141 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 707 of SEQ ID NO:141, b is an integer of 15 to 721, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:141, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 132**

The gene encoding the disclosed cDNA is thought to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

20 This gene is expressed primarily in adult lung, and to a lesser extent in the hypothalamus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pulmonary diseases and neurological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the pulmonary and respiratory systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. lung, brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such

a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in adult lung indicates that the protein product of this gene is useful for the diagnosis and treatment of pulmonary and respiratory disorders such as emphysema, pneumonia, and pulmonary edema and emboli. In addition, the tissue distribution indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:142 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1454 of SEQ ID NO:142, b is an integer of 15 to 1468, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:142, and where b is greater than or equal to a + 14.

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 133**

This gene is expressed primarily in human liver.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,

cirrhosis of the liver and other hepatic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. liver, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human liver indicates that the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:143 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 286 of SEQ ID NO:143, b is an integer of 15 to 300, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:143, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 134**

The gene encoding the disclosed cDNA is thought to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

5 This gene is expressed primarily in fetal kidney, and to a lesser extent in fetal liver and spleen.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, development and regeneration of liver and kidney and immunological disorders.

10 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive and excretory systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. kidney,

15 liver, spleen, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 382 as residues: Pro-70 to Arg-77, Tyr-102 to Thr-107.

The tissue distribution in fetal kidney indicates that the protein product of this gene is useful for the diagnosis and treatment of diseases of the kidney and liver, such as cirrhosis, kidney failure, kidney stones, and liver failure, hepatoblastoma, jaundice,

25 hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells. In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma. Protein, as well as, antibodies directed against the protein may show utility

30 as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are



related to SEQ ID NO:144 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2229 of SEQ ID NO:144, b is an integer of 15 to 2243, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:144, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 135**

This gene is expressed primarily in brain, bone marrow, and to a lesser extent in placenta, T cell, testis and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative and immunological diseases and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., CNS, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 383 as residues: Met-1 to His-6.

The tissue distribution in brain indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral

disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of hematopoietic disorders. This gene product is expressed in hematopoietic cells and tissues, suggesting that it plays a role in the survival, proliferation, and/or differentiation of hematopoietic lineages. Expression of this gene product in T cells and neutrophils also strongly indicates a role for this protein in immune function and immune surveillance.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:145 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1068 of SEQ ID NO:145, b is an integer of 15 to 1082, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:145, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 136**

The translation product of this gene is homologous to the human WD repeat protein HAN11, which is thought to function in signal transduction pathways.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MSLHGKRKEIYKYEAPWTVYAMNWSVRPDKRFRLALGSFVEEYNNKVQLV  
GLDEESSEFICRNTFDHPYPTTKLMWIPDTKGVYPDLLATSGDYLRVWRVGE  
TETRLCLNNNKNNSDFCAPLTSFDWNEVDPYLLGTSSIDTTCTIWGLETGQV

LGRVNLVSGHVKTQLIAHDKEVYDIAFSRAGGGRDMFASVGADGSVRMFDL  
 RHLEHSTIIYEDPQHHP LLRLCWNKQDPNYLATMAMDGMEVVILDVRVPAH  
 LXP GTTIEHVSMALLGPHIHPATSALQRMTTRLSSGTSSKCPEPLRTLSWPTQL  
 XGEINNVQWASTQPELSPSATT TAWRYSECSVGGAVPTRQGLLYFLPLPHPQS

- 5 (SEQ ID NO:1008),  
 MSLHGKRKEIYKYEAPWTVYAMNWSVRPDKRFRLALGSFVEEYNNKVQLV  
 GLDEESSEFICRNTFDHPYPTTKLMWIPDTKGVYPDLLATSGDYLRVWRVGE  
 TETRLECLNNNKNSDFCAPLTSFDWNEVDPYLL (SEQ ID NO:1009),  
 SFDWNEVDPYLLGTSSIDTTCTIWGLETGQVLGRVNLVSGHVKTQLIAHDKE  
 10 VYDIAFSRAGGGRDMFASVGADGSVRMFDLRHLEHSTIIYEDPQHHP LLRLC  
 WNKQDPNYLATMAMDGMEVVILDVRVPAHLXP GTTI (SEQ ID NO:1010),  
 and/or VGADGSVRMFDLRHLEHSTIIYEDPQHHP LLRLCWNKQD  
 PNYLATMAMDGMEVVILDVRVPAHLXP GTTIEHVSMALLGPHIHPATSALQR  
 MTRLSSGTSSKCPEPLRTLSWPTQLXGEINNVQWASTQPELSPSATT TAWRY  
 15 SECSVGGAVPTRQGLLYFLPLPHPQS (SEQ ID NO:1011). Moreover, fragments  
 and variants of these polypeptides (such as, for example, fragments as described  
 herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%  
 identical to these polypeptides and polypeptides encoded by the polynucleotide which  
 hybridizes, under stringent conditions, to the polynucleotide encoding these  
 20 polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides  
 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome  
 17. Accordingly, polynucleotides related to this invention are useful as a marker in  
 25 linkage analysis for chromosome 17.

This gene is expressed primarily in placenta, embryo, T cell and fetal lung,  
 and to a lesser extent in endothelial, tonsil and bone marrow.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 30 and for diagnosis of diseases and conditions which include, but are not limited to,  
 immunological and developmental diseases in addition to cancers. Similarly,  
 polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 384 as residues: Gly-19 to Gln-28, Pro-36 to Phe-42.

The tissue distribution in tumors of colon, ovary, and breast origins indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may also be used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:146 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4299 of SEQ ID NO:146, b is an integer of 15 to 4313, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:146, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 137**

This gene is expressed primarily in TNF and INF induced epithelial cells, T cells and kidney.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, inflammatory conditions particularly inflammatory reactions in the kidney. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of renal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. kidney, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 385 as residues: Thr-67 to Gly-72, Gln-132 to Ala-145, Arg-150 to Pro-157.

The tissue distribution in TNF and INF induced epithelial cells indicates that the protein products of this gene are useful for treating the damage caused by inflammation of the kidney. Furthermore, the tissue distribution in kidney indicates that this gene or gene product is useful in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:147 and may have been publicly available prior to conception

of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
 5 formula of a-b, where a is any integer between 1 to 1169 of SEQ ID NO:147, b is an integer of 15 to 1183, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:147, and where b is greater than or equal to a + 14.

## 10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 138**

The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1. (See Genbank Accession No. D63485).

15 This gene is expressed primarily in breast cancer and colon cancer, and to a lesser extent in thymus and fetal spleen.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
 20 cancers, especially of the breast and colon tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain  
 25 tissues or cell types (e.g. breast, colon, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

30 The tissue distribution in tumors of colon and breast origins indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as

well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:148 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 720 of SEQ ID NO:148, b is an integer of 15 to 734, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:148, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 139**

The gene encoding the disclosed cDNA is thought to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

This gene is expressed primarily in CD34 positive cells, and to lesser extent in activated T-cells and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune related diseases and hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such

a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in CD34 positive cells, T-cells and neutrophils indicates that the protein product of this gene is useful for the treatment and diagnosis of hematopoietic disorders and immune related diseases, such as anemia, leukemia, inflammation, infection, allergy, immunodeficiency disorders, arthritis, asthma, immune deficiency diseases such as AIDS. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Expression of this gene product in T cells and neutrophils also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:149 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1391 of SEQ ID NO:149, b is an integer of 15 to 1405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where b is greater than or equal to a + 14.



## FEATURES OF PROTEIN ENCODED BY GENE NO: 140

This gene was recently published by another group, who called the gene KIAA0313 gene. (See Genbank Accession No. d1021609.)

- 5 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:
- LYATATVISSPSTEXLSQDQGDRASLDAADSGRGSWTSCSSGSHDNIQTIQHQ  
 RSWETLPFGHTHFDYSGDPAGLWASSSHMDQIMFSDHSTKYNRQNSRESLE  
 QAQSRASWASSTGYWGEDSEGDTGTIKRRGGKDV SIEAESSSLTSVTTEETKP  
 10 VPMPAHIAVASSTTKGLIARKEGRYREPPPTPPGYIGIPITDFPEGHSH PARKPP  
 DYNVALQRSRMVARSSDTAGPSSVQQPHGHPTSSRPVNKPQWHKXNESDPRLAP  
 LAPIYQSQGFSTEEDEDEQVSAV (SEQ ID NO:1012),  
 HMDQIMFSDHSTKYNRQNSRESLEQAQSRASWASSTGYWGE (SEQ ID  
 NO:1013),  
 15 SVTTEETKPVMPAHIAVASSTTKGLIARKEGRYREPPPTPPGYIGIPITD (SEQ  
 ID NO:1014), and/or  
 VALQRSRMVARSSDTAGPSSVQQPHGHPTSSRPVNKPQWHKXNESDPRLAP  
 YQSQGF (SEQ ID NO:1015). Moreover, fragments and variants of these  
 polypeptides (such as, for example, fragments as described herein, polypeptides at  
 20 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides  
 and polypeptides encoded by the polynucleotide which hybridizes, under stringent  
 conditions, to the polynucleotide encoding these polypeptides ) are encompassed by  
 the invention. Antibodies that bind polypeptides of the invention are also  
 encompassed by the invention. Polynucleotides encoding these polypeptides are also  
 25 encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 4. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 4. (See Genbank Accession No. AB002311 ).

- 30 This gene is expressed primarily in ovarian cancer, tumors of the Testis, brain, and colon.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

and for diagnosis of diseases and conditions which include, but are not limited to, ovarian, testicle, brain and colon cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the male and female reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, testis, colon, ovary, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in tumors of colon, ovary, testis, and brain origins indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:150 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2876 of SEQ ID NO:150, b is an integer of 15 to 2890, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:150, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 141**

The gene encoding the disclosed cDNA is thought to reside on chromosome 18. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 18.

This gene is expressed primarily in spleen and colon cancer.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, colon cancer and immunological disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for  
10 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal tract and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. spleen, colon, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid  
15 and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in colon tumors indicates that the protein product of this gene is useful for the diagnosis and intervention of such tumors, in addition to  
20 other tissues and cell types where expression has been indicated. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be  
25 also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and  
30 graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In

addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:151 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2385 of SEQ ID NO:151, b is an integer of 15 to 2399, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 142**

The translation product of this gene is homologous to a T cell translocation protein, a putative zinc finger factor (See Genbank Accession No. 340454), as well as to the G-protein coupled receptor TM5 consensus polypeptide (See Genbank Accession No. R50734).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: CLLFVVFVSLGMRCLFWTIVYNVLYLKHKCNTVLLCYHLCSI (SEQ ID NO:1016), and/or ACSKLIPAFEMVMRAKDNVYHLDCFACQLCNQRXCVGDKFFLKNNXXLCQ TDYEEGLMKEGYAPXVR (SEQ ID NO:1017). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein,

polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal brain, and to a lesser extent in frontal cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological disorders, including brain cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the Central Nervous System, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal brain indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states and behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo. Furthermore, elevated expression of this gene product within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's.

Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:152 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 788 of SEQ ID NO:152, b is an integer of 15 to 802, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:152, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 143**

The translation product of this gene has significant homology to the Fas ligand, which is a cysteine-rich type II transmembrane protein/tumor necrosis factor receptor homolog. Mutations within this protein have been shown to result in generalized lymphoproliferative diseases leading to the development of lymphadenopathy and autoimmune disease (See Medline Article No. 94185175).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

**SALSEPGAPDRRRPCPESVPRRPDDEQWPPPTALCLDVAPLPPSS (SEQ ID**

**NO:1018).** Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention. (See Genbank Accession No. 473565).

This gene is expressed primarily in osteoblasts, lung, and brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, osteoblast-related, pulmonary, neurological, and immunological diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal and nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. lung, brain, skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 391 as residues: Trp-33 to Thr-40, Lys-45 to Ile-63.

The tissue distribution in osteoblasts, lung, and brain, combined with its homology to the Fas ligand, indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues. Because the Fas ligand gene is known to be expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including asthma, immune deficiency diseases such as AIDS and leukemia, and various autoimmune disorders including lupus and arthritis. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:153 and may have been publicly available prior to conception

of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

5 formula of a-b, where a is any integer between 1 to 447 of SEQ ID NO:153, b is an integer of 15 to 461, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:153, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 144

This gene shares sequence homology with a 21.5 KD transmembrane protein in the SEC15-SAP4 intergenic region of yeast. (See Genbank Accession No. 1723971.)

15 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: PVGYLDKQVPDTSVQETDRILVEKRCWDIALGPLKQIPMNLF (SEQ ID NO:1019),

AHASESGERWWACCGVRFLRSIEAIGRSCCHDGPGLVANRGRFRKWAIEL

20 SGPGGGSRGRSDRGSGQGDSLYPVGYLDKQVPDTSVQETDRILVEKRCWDIALGPLKQIPMNLFIMYMAGNTISIFPTMMVCMMAWRPIQALMAISATFKMLES

SSQKFLQGLVYLIGNLMGLALAVYKCQSMGLLPHTASDWLAFIEPPERMEFS

GGGLLL (SEQ ID NO:1020), PVGYLDKQVPDTSVQETDRILVEKRCWDIALGPLKQIPMNLF (SEQ ID NO:1022), and/or

25 ATFKMLESSQKFLQGLVYLIGNLMGLALAVYKCQSMGLLPHTASD (SEQ ID NO:1021). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

30 encoding these polypeptides ) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.



This gene is expressed primarily in osteoclastoma, hemangiopericytoma, liver, lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, osteoclastoma, hemangiopericytoma, liver and lung tumors. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the above tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the lung and liver systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. lung, liver, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of tumors of the osteoclastoma, hemangiopericytoma, liver and lung, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:154 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2374 of SEQ ID NO:154, b is an integer of 15 to 2388, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:154, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 145

The translation product of this gene shares homology with the glucagon-69  
 5 gene which may indicate this gene plays a role in regulating metabolism. (See  
 Genbank Accession No. A60318)

In specific embodiments, polypeptides of the invention comprise, or  
 alternatively consists of, an amino acid sequence selected from the group:

PTTKLDIMEKKKHIQIRFPSFYHKLVDSGRMRSKRETRREDSDTKHNL (SEQ  
 10 ID NO:1023), FLWKSLLLRIFYFKMRQH (SEQ ID NO:1024), and/or  
 YHYLLSSFLSYSSSSQNLVPYGRKMGTLFECVFFFP (SEQ ID NO:1025).

Moreover, fragments and variants of these polypeptides (such as, for example,  
 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
 15 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides) are encompassed by the invention. Antibodies that bind  
 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
 encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in brain, kidney, colon, and testis.

20 Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 brain, kidney, colon, and testicular cancer. Similarly, polypeptides and antibodies  
 directed to these polypeptides are useful in providing immunological probes for  
 25 differential identification of the tissue(s) or cell type(s). For a number of disorders of  
 the above tissues or cells, particularly of the male reproductive system, neurological,  
 circulatory, and gastrointestinal systems, expression of this gene at significantly  
 higher or lower levels may be routinely detected in certain tissues or cell types (e.g.  
 brain, kidney, colon, testis, cancerous and wounded tissues) or bodily fluids (e.g.,  
 30 lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell  
 sample taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in brain, kidney, colon, and testis origins, indicates that the protein product of this gene is useful for the diagnosis and intervention of tumors

of these tissues. The protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions

such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies,

neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and

infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia,

obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses ,

autism, and altered behaviors, including disorders in feeding, sleep patterns, balance,

and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene

product is involved in synapse formation, neurotransmission, learning, cognition,

homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene

product may also play a role in the treatment and/or detection of developmental

disorders associated with the developing embryo, sexually-linked disorders, or

disorders of the cardiovascular system. Protein, as well as, antibodies directed against

the protein may show utility as a tumor marker and/or immunotherapy targets for the

above listed tissues. Protein, as well as, antibodies directed against the protein may

show utility as a tumor marker and/or immunotherapy targets for the above listed

tumors and tissues. The tissue distribution indicates that the protein product of this

gene is useful for the detection/treatment of neurodegenerative disease states and

behavioral disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's

Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder

and panic disorder. In addition, the gene or gene product may also play a role in the

treatment and/or detection of developmental disorders associated with the developing

embryo, sexually-linked disorders, or disorders of the cardiovascular system.

Many polynucleotide sequences, such as EST sequences, are publicly

available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:155 and may have been publicly available prior to conception

of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

5 formula of a-b, where a is any integer between 1 to 628 of SEQ ID NO:155, b is an integer of 15 to 642, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:155, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 146

The translation product of this gene shares sequence homology with goliath protein, which is a Drosophila protein thought to be important in the regulation of gene expression during development. Protein may serve as a transcription factor.

15 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

TEHIIAVMITELRGKDILSYLEKNISVQMTIAVGTRMPPKNFSRGS�VFVSISFI  
VLMISSAWLIFYFIQKIRYTNARDRNQRR LGDAAKKAISKLTTRTVKKGDKE  
TDPDFDHCAVCIESYKQNDVVRILPCKHVFHKSCVDPWLSEHCTCPMCKLNI

20 LKALGIV (SEQ ID NO:1026),

MTHPGTEHIIAVMITELRGKDILSYLEKNISVQMTIAVGTRMPPKNFSRGS�VF  
VSISFIVLMISSAWLIFYFIQKIRYTNARDRNQRR LGDAAKKAISKLTTRTVKK  
GDKETDPDFDHCAVCIESYKQNDVVRILPCKHVFHKSCVDPWLSEHCTCPMC  
KLNILKALGIVPNLPCTDNVAFDMERLTRTQAVNRRSALGDLAGDNSLGLEP

25 LRTSGISPLPQDGELTPRTGEINIAVTKEWFIIASFGLLSALTLCYMIIRATASLN  
ANEVEWF (SEQ ID NO:1027),

TEHIIAVMITELRGKDILSYLEKNISVQMTIAVGTRMPPKNFSRGS�VFVSISFI  
VLMISSAWLIFYF (SEQ ID NO:1028),

30 SISFIVLMISSAWLIFYFIQKIRYTNARDRNQRR LGDAAKKAISKLTTRTVKKG  
DKE (SEQ ID NO:1029),

VKKGDKETDPDFDHCAVCIESYKQNDVVRILPCKHVFHKSCVDPWLSEHCTC  
PMCKLNILKALGIV (SEQ ID NO:1030),

AVGTRMPPKNFSRGS LVFVSISFIVLMISSAWLIFYFIQKIRYTNARDRNQRR.  
GDAAKKAISKLTTRT (SEQ ID NO:1031),

5 SCVDPWLSEHCTCPMCKLNILKALGIVPNLPC (SEQ ID NO:1032),

PLHGVADHLGCDPOTRFFVPPNIKQWLALLQRGNCTFKEKISRAAFHNAVAV

10 MPPKNFSRGSLVFVSISFIVLMISSAWLIFYFIQKIRYTNARDRNQRRRLGDAAK

DPWLSEHCTCPMCKLNILKALGIVPNLPCTDNVAFDMERLTRTQAVNRRSAL

TLCYMIIRATASLNANEVEWF (SEQ ID NO:1034), and/or

YNNKSKEE (SEQ ID NO:1035). Moreover, fragments and variants of these

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides

20 conditions, to the polynucleotide encoding these polypeptides) are encompassed by

encompassed by the invention. Polynucleotides encoding these polypeptides are also

When tested against Jurkat cell lines, supernatants removed from cells

activates T-cells through the Jak-STAT signal transduction pathway. The gamma

which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large,

30 Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS

differentiation of cells.

This gene is expressed primarily in macrophage, breast, kidney and to a lesser extent in synovium, hypothalamus and rhabdomyosarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, schizophrenia and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and neural system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, kidney, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in macrophage, hypothalamus, and kidney, combined with the homology to a zinc finger protein indicates that the protein product of this gene is useful for the treatment of schizophrenia, kidney disease and other cancers. Furthermore, the tissue distribution in macrophage, breast, and kidney origins indicates that the protein product of this gene is useful for the diagnosis and intervention of tumors within these tissues, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:156 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1237 of SEQ ID NO:156, b is an integer of 15 to 1251, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:156, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 147

The translation product of this gene shares sequence homology with HNP36 protein, an equilibrative nucleoside transporter, which is thought to be important in gene transcription as well as serving as an important component of the nucleoside transport apparatus (See Genbank Accession No. 1845345).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MSGQGLAGFFASVAMICAIASGSELSESAFGYFITACAVIILTIICYLGLP  
RLEFYRYYYQQLKLEGPGEQETKLDLISKGEEPRAGKEESGVSVSNSQPTNESH  
SIKAILKNISVLAFSVCFTITIGMFPAVTVEVKSSIAGSSTWERYFIPVSCFLT  
FNI  
FDWLGRSLTAVFMWPGKDSRWLPSWXLARLVFVPLLLLCNIKPRRYLTVV  
FEHDAWFIFFMAAFASNGYLASLCMCFGPKKVKPAEAETAEPSPWSSCVW  
VWHWGLFSPSCSGQLCDKGWTEGLPASLPVCLLPLPSARGDPEWGGFFF (SEQ  
ID NO:1036),  
MSGQGLAGFFASVAMICAIASGSELSESAFGYFITACAVIILTIICYLGLPRLEF  
YRYYYQQLKLEGPGEQETKLDLISKGEEPRAGKEESGVSVSNSQPTNESH  
SI  
(SEQ ID NO:1037),  
SGVSVSNSQPTNESH  
SIKAILKNISVLAFSVCFTITIGMFPAVTVEVKSSIAGS  
STWERYFIPVSCFLT  
FNI  
FDWLGRS (SEQ ID NO:1038),  
TIGMFPAVTVEVKSSIAGSSTWERYFIPVSCFLT  
FNI  
FDWLGRSLTAVFMWPG  
KDSRWLPSWXLARLVFVPLLLLCNIKPRRYLTVVFEHDA (SEQ ID NO:1039),  
and/or  
FGPKKVKPAEAETAEPSPWSSCVWVWHWGLFSPSCSGQLCDKGWTEGLPAS  
LPVCLLPLPSARGDPEWGGFFF (SEQ ID NO:1040). Moreover, fragments and

variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

An additional embodiment is the polynucleotide fragments encoding these polypeptide fragments. The gene encoding the disclosed cDNA is thought to reside on chromosome 6. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 6.

This gene is expressed primarily in eosinophils and aortic endothelium, and to a lesser extent in umbilical vein endothelial cell and thymus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hemopoietic disease. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the circulatory system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. circulatory, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution eosinophils and aortic endothelium, combined with the homology to the HNP36 protein indicates that the protein product of this gene is useful for the treatment of blood neoplasias and other hemopoietic disease.

Furthermore, elevated expression of this gene product by endothelial cells indicates that it may play vital roles in the regulation of endothelial cell function; secretion; proliferation; or angiogenesis. Protein, as well as, antibodies directed against the



protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:157 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2113 of SEQ ID NO:157, b is an integer of 15 to 2127, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:157, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 148**

The gene encoding the disclosed cDNA is thought to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

This gene is expressed primarily in breast cancer cell lines, thymus stromal cells, and ovary.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, endocrine and female reproductive system diseases including breast cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the endocrine system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. thymus, ovary, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having

such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in breast cancer cells and ovary indicates that the protein product of this gene is useful for the diagnosis and treatment of endocrine disorders. In addition, the tissue distribution in tumors of thymus, ovary, and breast origins indicates that the protein product of this gene is useful for diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tumors and tissues

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:158 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1611 of SEQ ID NO:158, b is an integer of 15 to 1625, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:158, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 149**

The translation product of this gene has homology to pmt1 and pmt 2, two conserved *Schizosaccharomyces pombe* genes.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: DDDGFEIVPIEDPAKHRILDPEGLALGAVIASSKKAKRDLIDNSFNRYTFNEDE  
GELPEWVFVQEEKQHRIRQLPVGKKEVEHYRKRWREINARPIXXXXXXXXXXXX  
XXXXXXXXLEQTRKKAEAVVNTVDIXRTRES (SEQ ID NO:1041), DDDG  
FEIVPIEDPAKHRILDPEGLALGAVIASSKKAKRDLIDNSFNRYTF (SEQ ID

NO:1042), and/or

KRWREINARPIXXXXXXXXXXXXXXXXXXXXXLEQTRKKAEAVVNTVDIXRTRES  
(SEQ ID NO:1043). Moreover, fragments and variants of these polypeptides (such  
as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
5 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides) are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding these polypeptides are also encompassed by the  
10 invention. (See Genbank Accession No. e1216734).

This gene is expressed primarily in retina and ovary, and to a lesser extent in  
breast cancer cells, epididymus and osteosarcoma.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
15 and for diagnosis of diseases and conditions which include, but are not limited to,  
neuronal growth disorders, cancer and reproductive system disorders. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s).  
For a number of disorders of the above tissues or cells, particularly of the neural and  
20 reproductive system, expression of this gene at significantly higher or lower levels  
may be routinely detected in certain tissues or cell types (e.g. retina, ovary,  
reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum,  
plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken  
from an individual having such a disorder, relative to the standard gene expression  
25 level, i.e., the expression level in healthy tissue or bodily fluid from an individual not  
having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID  
NO: 397 as residues: Met-1 to Gly-7.

The tissue distribution in ovary, breast cancer cells, and epididymus indicates  
30 that the protein product of this gene is useful for the diagnosis or treatment of  
reproductive system diseases and cancers, in addition to other tumors where  
expression has been indicated. Protein, as well as, antibodies directed against the

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protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:159 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1673 of SEQ ID NO:159, b is an integer of 15 to 1687, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:159, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 150**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MIKDKGRARTALTSSQPAHLCPENPLLHLKAAVKEKKRNKKKKKTIGSPKRIQS  
PLNNKLLNSPAKTLPGACGSPQKLIDGFLKHEGPPAEKPLEELSASTSGVPGLS  
SLQSDPAGCVRPPAPNLAGAVEFNDVKTLLEWITTISDPMEEDILQVVKYCT  
DLIEEKDLEKLDLVIKYMKRLMQQSVESVWNMAFDNFILDNVQVVLQQTYGS  
TLKVT (SEQ ID NO:1044),

MIKDKGRARTALTSSQPAHLCPENPLLHLKAAVKEKKRNKKKKKTIGSPKRIQ  
(SEQ ID NO:1045),

KRIQSPLNNKLLNSPAKTLPGACGSPQKLIDGFLKHEGPPAEKPLEELSASTSG  
VPGLSSLQSDPAGCVRPPAPNLAGAVEFNDVKTLLEWITTI SDPM (SEQ ID  
NO:1046),

TISDPMEEDILQVVKYCTDLIEEKDLEKLDLVIKYMKRLMQQSVESVWNMAF  
DFILDNVQVVLQQTYGSTLKVT (SEQ ID NO:1047),

VCCKTTWTLRIKSNAIFQTDSTDCISLFMYFITRSSFSSIRSVQYFTTW  
RMSSSIGSEIVVIHSLSKVFTSLNSTAPARLGAGGLTQPAGSDCKLERPGTPEV

EAESSSRGFSAGGPSCFRNPSINFWGLPQAPGRVFAGLLSSLLFKGL (SEQ ID NO:1048), WTLSRIKSNAIFQTDSTDCCISLFM (SEQ ID NO:1049), FTTWRMSSSIGSEIVVIHSLSKVFTSLNSTAPARLGA (SEQ ID NO:1050), and/or GGPSCFRNPSINFWGLPQAPGRVFAGLL (SEQ ID NO:1051). Moreover,

5 fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides  
10 of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

15 This gene is expressed primarily in 12 week embryo, and to a lesser extent, in hemangiopericytoma and frontal cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
20 developmental or neural disorders, particularly hemangiopericytoma, and other proliferative conditions, including cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural system and developing systems,  
25 expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
30 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 398 as residues: Leu-4 to Lys-11.

The tissue distribution in embryonic and neural tissues indicates that the protein product of this gene is useful for the treatment of growth disorders, hemangiopericytoma and other soft tissue tumors. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:160 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1828 of SEQ ID NO:160, b is an integer of 15 to 1842, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:160, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 151

The translation product of this gene has been found to have homology to a  
 5 human DNA mismatch repair protein PMS3 (See Genbank Accession No. R95250).

In specific embodiments, polypeptides of the invention comprise, or  
 alternatively consists of, an amino acid sequence selected from the group:  
 FCHDCKFPEASPAMNCEP (SEQ ID NO:1052), FCHDCKFPEASPAMNCEP  
 (SEQ ID NO:1053), and/or HEPYAVLVI (SEQ ID NO:1054). Moreover, fragments  
 10 and variants of these polypeptides (such as, for example, fragments as described  
 herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%  
 identical to these polypeptides and polypeptides encoded by the polynucleotide which  
 hybridizes, under stringent conditions, to the polynucleotide encoding these  
 polypeptides) are encompassed by the invention. Antibodies that bind polypeptides  
 15 of the invention are also encompassed by the invention. Polynucleotides encoding  
 these polypeptides are also encompassed by the invention.

This gene is expressed primarily in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 20 and for diagnosis of diseases and conditions which include, but are not limited to,  
 immune or hematopoietic disorders, such as lymphoma, immunodeficiency diseases,  
 and cancers resulting from genetic instability. Similarly, polypeptides and antibodies  
 directed to these polypeptides are useful in providing immunological probes for  
 differential identification of the tissue(s) or cell type(s). For a number of disorders of  
 25 the above tissues or cells, particularly of the immune system, expression of this gene  
 at significantly higher or lower levels may be routinely detected in certain tissues or  
 cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or  
 bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or  
 another tissue or cell sample taken from an individual having such a disorder, relative  
 30 to the standard gene expression level, i.e., the expression level in healthy tissue or  
 bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 399 as residues: Met-1 to Lys-6.

The tissue distribution in neutrophils, combined with the sequence homology to a human mismatch DNA repair enzyme indicates that the protein product of this gene is useful for diagnosis of Hodgkin's lymphoma, since the elevated expression and secretion by the tumor mass may be indicative of tumors of this type.

Additionally the gene product may be used as a target in the immunotherapy of the cancer. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Furthermore, its homology to a known DNA repair protein would suggest the gene may be useful in establishing cancer predisposition and prevention or be of use in gene therapy applications. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:161 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 756 of SEQ ID NO:161, b is an integer of 15 to 770, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:161, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 152**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:  
PQPSNFPTTVRNLPYSGAGAQPPTSNC (SEQ ID NO:1055). Moreover, fragments



and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune or hematopoietic disorders, such as infectious diseases and lymphoma. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the treatment of inflammation and infectious diseases. Expression of this gene product in neutrophils indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such

as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:162 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 505 of SEQ ID NO:162, b is an integer of 15 to 519, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:162, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 153**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MASSVPAGGHTRAGGIFLIGKLDLEASLFKSFQWLPFVLRKKCNFFCWDSSA  
HSLPLHPLSASCSAPACHASDTHLLYPSTRALCPSIFAWLVAPHSVFRTNAPGP  
TPSSQSSPVFPVFPVSFIMALIVCXLVCC (SEQ ID NO:1056),  
MASSVPAGGHTRAGGIFLIGKLDLEASLFKSFQWLPFVLRKKCNFFCWDSSA  
HSLPLHPLSASCSAPACHA (SEQ ID NO:1057),

MASSVPAGGHTRAGGIFLIGKLDLEASLFKSFQWLPFVLRKKCNFFCWDSSA  
HSLPLHPLSASCAPACHASDTHLLYPSTRALCPSIFAWLVAPHSVFRTNAPGP

these polypeptides are also encompassed by the invention.

Polynucleotides and polypeptides of the invention are useful as reagents for

disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 401 as residues: Ser-11 to Pro-17.

The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the treatment of infectious diseases and inflammation. Moreover, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lens tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:163 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 739 of SEQ ID NO:163, b is an

integer of 15 to 753, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:163, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 154

This gene is primarily expressed in ovary, uterus, adipose tissue, brain, and the liver.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, neural, hepatic, and metabolic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., ovary, uterus, adipose tissue, brain, liver, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 402 as residues: Asn-56 to Gly-67.

The tissue distribution of this gene product in ovary and uterus indicates that the protein product of this gene is useful for diagnostic or therapeutic uses in the treatment of the female reproductive system, obesity, and liver disorders, particularly cancer in the above tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:164 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1879 of SEQ ID NO:164, b is an integer of 15 to 1893, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:164, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 155**

The gene encoding the disclosed cDNA is believed to reside on chromosome  
3. Accordingly, polynucleotides related to this invention are useful as a marker in  
15 linkage analysis for chromosome 3.

This gene is expressed in multiple tissues including brain, aortic endothelial cells, smooth muscle, pituitary, testis, melanocytes, spleen, neutrophils, and placenta.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
20 and for diagnosis of diseases and conditions which include, but are not limited to, immunological or vascular disorders, including immunodeficiencies, cancers of the brain and the female reproductive system, as well as cardiovascular disorders, such as atherosclerosis and stroke. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
25 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, vascular, endothelial, neural, hematopoietic, reproductive, integumentary, placental, endocrine, and cancerous and wounded  
30 tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having

such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neural tissue indicates that the protein product of this gene is useful in the treatment/detection of disorders in the nervous system, including schizophrenia, neurodegeneration, neoplasia, brain cancer as well as vascular and female reproductive disorders, including cancer within the above tissues. Moreover, the protein product of this gene may also be useful in the treatment and/or detection of other vascular disorders which include, but are not limited to, aneurysms, emboli, thrombosis, atherosclerosis, microvascular disease, or stroke. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:165 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2139 of SEQ ID NO:165, b is an integer of 15 to 2153, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:165, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 156**

The translation product of this gene shares sequence homology with the human gene encoding cytochrome b561 (See Genbank Accession No. P10897). Cytochrome b561 is a transmembrane electron transport protein that is specific to a subset of secretory vesicles containing catecholamines and amidated peptides. This protein is thought to supply reducing equivalents to the intravesicular enzymes dopamine-beta-hydroxylase and alpha-peptide amidase.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MAMEGYWRFLALLGSALLVGFLSVIFALVWVLHYREGLGWDGSALEFNWH  
PVLMTGTFVFIQGLAIVYRLPWTWKCSKLLMKSIHAGLNAVAAILAISVVAV  
5 FENHNVNNIANMYSLHSWVGLIAVICYLLQLLSGFSVFLLPWAPLSLRAFLMP  
IHVYSGIVIFGTVIATALMGLTEKLIFSLRDPAYSTFPPEGVFVNTLGLLLVFG  
ALIFWIVTRPQWKRPKEPNSTILHPNGGTEQGARGSMPAYSGNNMDKSDSEL  
NSEVAARKRNALDEAGQRSTM (SEQ ID NO:1063),

AHASAHASGGAEYGAL (SEQ ID NO:1064),

10 QYSQYVQSAQLGWTDSCHMLFVTASFRFFSLSASMGSASFSPSISHAHTCLFW  
NCHLWNSDCNSTYGIDRETDFPERSCIQYIPARRCFRKYAWPSDPGVRGPHF  
LDHQATAMETS (SEQ ID NO:1065), ASMG

AFSPSISHAHTCLFWNCHLWNSDCNSTYG (SEQ ID NO:1066),

FVHVVARVGWHGTSCSLFSASIWMKNGRIWLLRTFPLRSGDYPKNEGPEHQ

15 DQKAKRIYENTFWRECTVCRISQGKNQFLCQSHKCCCNHCSKDDNSRINMY  
GHEKCSERKRSPWKQKD (SEQ ID NO:1067), and/or

ASIWMKNGRIWLLRTFPLRSGDYPKNEGPEHQ (SEQ ID NO:1068). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or

20 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

25 The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in anergic T-cells.

30 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune or hematopoietic disorders, and metabolic related diseases. Similarly,



polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be

5 routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, metabolic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not  
10 having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 404 as residues: Pro-222 to Asn-231, Asn-238 to Gly-247, Ala-251 to Leu-264, Ala-280 to Thr-285.

The tissue distribution in anergic T-cells indicates that the protein product or  
15 mRNA of this gene is useful for the treatment or diagnosis of immune system and metabolic diseases or conditions including Tay-Sachs disease, phenylketonuria, galactosemia, various porphyrias, and Hurler's syndrome. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:166 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
25 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1237 of SEQ ID NO:166, b is an integer of 15 to 1251, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:166, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 157**

The translation product of this gene shares sequence homology with collagen which is important in mammalian development. This gene also shows sequence homology with bcl-2 and the HNK-1 sulfotransferase of *Rattus norvegicus* which is thought be involved in carbohydrate biosynthesis. (See Genbank Accession No. P80988 and AF022729, respectively.) When tested against Jurkat cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates T-cells through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:  
 PGRAGPSPGLSLQLPAEPGHPAGNLAPLTSRPQPLCRIPAVPG (SEQ ID NO:1069). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

This gene is expressed primarily in HL-60 tissue culture cells, and to a lesser extent, in liver, breast, and uterus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunological diseases, hereditary disorders involving the MHC class of immune molecules, as well as developmental disorders and reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and reproductive system expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, hepatic, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 405 as residues: Ser-39 to Gly-46, Leu-49 to Ala-62.

The tissue distribution in reproductive, and immune tissues, combined with the homology to collagen and the detected GAS biological activity indicates that the protein product of this gene is useful for diagnosis and treatment of hereditary MHC disorders and particularly autoimmune disorders including rheumatoid arthritis, lupus, scleroderma, and dermatomyositis, as well as many reproductive disorders, including cancer of the uterus, and breast tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:167 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 868 of SEQ ID NO:167, b is an integer of 15 to 882, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:167, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 158

This gene is expressed primarily in the amygdala region of the brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, a variety of brain disorders, particularly those effecting mood and personality, in addition to neurodegenerative conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in the amygdala indicates that the protein product of this gene is useful for the treatment and/or diagnosis of a variety of brain disorders, particularly bi-polar disorder, uni-polar depression, and dementia. Moreover, The tissue distribution indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role

in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:168 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1194 of SEQ ID NO:168, b is an integer of 15 to 1208, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:168, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 159**

This gene is expressed in a variety of tissues and cell types including brain,  
20 smooth muscle, kidney, salivary gland, and T-cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neural, renal, vascular, metabolic, or immune disorders, particularly cancers.

25 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous, urinary, salivary, digestive, and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain  
30 tissues or cell types (e.g., neural, renal, vascular, metabolic, immune cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such

a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 407 as residues: Asp-43 to Asp-60.

5           The tissue distribution in brain; smooth muscle, and T-cells indicates that the protein product of this gene is useful for diagnosis of various neurological, and cardiovascular disorders, but not limited to cancer within the above tissues. Additionally the gene product may be used as a target in the immunotherapy of the cancer. Because the gene is expressed in cells of lymphoid origin, the natural gene  
10       product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:169 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
20       cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1244 of SEQ ID NO:169, b is an integer of 15 to 1258, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:169, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 160**

30           The translation product of this gene shares sequence homology with collagen, which is thought to be important in cellular interactions, extracellular matrix formation, and has been found to be an identifying determinant in autoimmune disorders. Moreover, this gene shows sequence homology with the yeast protein,

Sls1p, an endoplasmic reticulum component involved in the protein translocation process in the Yeast *Yarrowia lipolytica*. (See Genbank Accession No. 1052828; see also J. Biol. Chem. 271, 11668-11675 (1996).) In *Mus musculus*, this same region shows sequence homology with the heavy chain of kinesin. (See Genbank Accession No. 2062607.) Recently, suppression of the heavy chain of kinesin was shown to inhibit insulin secretion from primary cultures of mouse beta-cells. (See Endocrinology 138 (5), 1979-1987 (1997).) Moreover, kinesin was found associated with drug resistance and cell immortalization. (See Genbank Accession No. 468355.) Thus, it is likely that this gene also acts as a genetic suppressor element.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: ARGRRRGRLELWELCLPLGCRRRRSLTMAPQSLPSSRMAPLG (SEQ ID NO:1070),

NGQASTAKMSSCLRSPTLAPLSLTSGIPVQSWCGASSQLLQQAVDRAQQLL  
EVALVLTILQLQAGQHLVLSLQAGQCPAELGVLTVAVPAGGQEDAQCQLQHL  
LTGIMLGQRQEVGRDLAPALFPQAWQEVYLAILLQLLWGHLLGQLSLLLGEH  
LLRDQVVEQCDHAHGEHLRALLHQGPQDLQPPELQELPLGIGEVAQQGAQ  
CKQDLLLCSERLRGQDDQQLQGSPFDGLHLDLGVAGKGSAQHKRSILLHE  
GLCAVQPIDHHLKTTKGKQVLRIVHLMDIIFKIKERSNLLFQTGAGTIELVDQP  
YHDLHVSNDNIQLIKVFLQFLNGAEEPLYLSLPCLVFL (SEQ ID NO:1071),  
QHLVLSLQAGQCPAELGVLTVAVPAGGQEDAQC (SEQ ID NO:1072),  
QLSLLLGEHLLRDQVVEQCDHAHGEH (SEQ ID NO:1073), GS  
PFDGLHLDLGVAGKGSAQHKRSILLHEGLC (SEQ ID NO:1074), and/or  
HLMDII FKIKERSNLLFQTGAGTIELVDQP (SEQ ID NO:1075). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

This gene is expressed primarily in the greater omentum, and to a lesser extent in gall bladder, stromal bone marrow cells, lymph node, liver, testes, pituitary, and thymus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the endocrine, gastrointestinal, and immunological systems, including autoimmune disorders and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, metabolic, immune, hematopoietic, hepatic, reproductive, endocrine, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 408 as residues: Asn-27 to Leu-47, Gln-81 to Lys-88, Asp-93 to Lys-102, Asn-107 to Leu-116, Met-129 to Glu-141, Glu-150 to Asp-157, Lys-176 to Glu-185, Glu-333 to Tyr-349, Cys-393 to Leu-403, Gln-423 to Gly-429.

The tissue distribution within gastrointestinal, endocrine and immunological tissues, combined with the sequence homology to a conserved collagen motif, indicates that the protein product of this gene is useful for the diagnosis of various autoimmune disorders including, but not limited to, rheumatoid arthritis, lupus erythematosus, scleroderma, and dermatomyositis. Because the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders



including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:170 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1610 of SEQ ID NO:170, b is an integer of 15 to 1624, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:170, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 161**

This gene has homology to the tissue inhibitor of metalloproteinase 2. Such inhibitors are vital to the proper regulation of metalloproteins such as collagenases, which has implications for tissue regeneration and autoimmune disorders (See Genbank Accession No. P16368). When tested against Jurkat cell lines, supernatants removed from cells containing this gene activated the GAS (gamma activating sequence) promoter element. Thus, it is likely that this gene activates T-cells cells through the JAK-STAT signal transduction pathway. GAS is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells. In addition, this gene maps to chromosome 17, and therefore, may be used as a marker in linkage analysis for chromosome 17 (See Genbank Accession No. P16368).

This gene is expressed primarily in several types of cancers including osteoclastoma, chondrosarcoma, and rhabdomyosarcoma, and to a lesser extent, in non-malignant tissues including synovium, amygdala, testes, and placenta.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune or integumentary disorders, particularly cancers of bone and cartilage, as well as various autoimmune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the musculoskeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skeletal, integumentary, synovium, muscle, fibroids, reproductive, neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 409 as residues: Thr-24 to Thr-34.

The tissue distribution in various cancers, combined with the sequence homology to a collagenase inhibitor and the detected GAS biological activity, indicates that the protein product of this gene is useful for the detection of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. The expression of this gene product would also suggest a role in the detection and treatment of disorders and conditions afflicting the skeletal system, in particular osteoporosis, bone cancer, as well as, connective tissue disorders (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and

specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the  
 5 above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:171 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
 10 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1989 of SEQ ID NO:171, b is an integer of 15 to 2003, where both a and b correspond to the positions of nucleotide  
 15 residues shown in SEQ ID NO:171, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 162**

20 This gene is homologous to the mitochondrial ATP6 gene, and therefore is likely a homolog of this gene family (See Genbank Accession No. X76197).

This gene is expressed primarily in brain tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample  
 25 and for diagnosis of diseases and conditions which include, but are not limited to, neural disorders, including, but not limited to, neurodegenerative conditions, Down's syndrome, depression, Schizophrenia, and epilepsy. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of  
 30 disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, and cancerous and wounded

tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution in brain tissue indicates this gene is useful for diagnosis of various neurological disorders including, but not limited to, brain cancer. Additionally the gene product may be used as a target in the immunotherapy of cancer in the brain as well as for the diagnosis of metabolic disorders such as obesity, Tay-Sachs disease, phenylketonuria and Hurler's Syndrome. Similarly, the protein product

10 of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and

15 infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function.

20 Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies

25 directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:172 and may have been publicly available prior to conception

30 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 772 of SEQ ID NO:172, b is an integer of 15 to 786, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:172, and where b is greater than or equal to a + 14.

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## FEATURES OF PROTEIN ENCODED BY GENE NO: 163

The translation product of this gene was found to have homology to the MRS3 and 4 protein of *Saccharomyces cerevisiae* (See Genbank Accession No. gi|3996 ), which is known to suppress a splice defect in mitochondrial by possibly serving to modulate the cation-solute concentration in mitochondria.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

DEPCPPPAASCAPPSWRMELRTGSVGSQAVARRMDGDSRDGGGGKDATGSE  
DYENLPTSASVSTHMTAGAMAGILEHSVMYPVDSVKTRMQSLSPDPAQYTSIYGALKKIMRTEASGGPCEASTS (SEQ ID NO:1076),

RMELRTGSVGSQAVARRMDGDSRDGGGGKDATGS (SEQ ID NO:1077),  
and/or PVDSVKTRMQSLSPDPAQYTSIYGAL (SEQ ID NO:1078). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

This gene is expressed primarily in placenta, neutrophils, and microvascular endothelial cells, and to a lesser extent, brain, prostate, spleen, thymus, and bone.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune, vascular, or reproductive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, vascular, endothelial, reproductive, neural, skeletal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 164**

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in neutrophils, monocytes, bone marrow, and fetal liver.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system or hematopoietic disorders including, but not limited to, autoimmune disorders such as lupus, leukemia and immunodeficiency disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be

routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, hepatic, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, amniotic fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative  
 5 to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in various immune system tissues indicates that the protein product of this gene is useful for the diagnosis of various immunological disorders such as Hodgkin's lymphoma, arthritis, asthma, immune deficiency diseases  
 10 such as AIDS, and leukemia. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow  
 15 reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of  
 20 various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
 25 related to SEQ ID NO:174 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
 30 formula of a-b, where a is any integer between 1 to 1355 of SEQ ID NO:174, b is an integer of 15 to 1369, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:174, and where b is greater than or equal to a + 14.

# FEATURES OF PROTEIN ENCODED BY GENE NO: 165

5           The translation product of this gene shares sequence homology with dystrophin which is thought to be defective in both Duchene and Becker Muscular Dystrophy.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 10 MKLLGECSSSIDSVKRLEHKLKEEEESLPGFVNLHSTETQTAGVIDRWELLQA  
 QALSKELRMKQNLQKWQQFNSDLNSIWAWLGDTEEELEQLQRLELSTDIQTI  
 ELQIKKLKELQKAVDHRKAILLSINLCSPEFTQADSKESRDLQDRLXQMNGRW  
 DRVCSLLEEWGRLLQDALMQCQGFHEMSHGLLLMLENIDRRKNEIVPIDSNL  
 DAEILQDHHKQLMQIKHELLESQLRVASLQDMSCQLLVNAEGTDCLEAKEK  
 15 VHVIGNRLKLLLKEVSRHIKELEKLLDVSSSQDLSSWSSADELDTSGSVSPX  
 SGRSTPNRQKTPRGKCSLSQPGPSVSSPHSRSTKGGSDSSLSEXPGRSGRGFL  
 FRVLRAALPLQLLLLLLIGLACLVPMSEEDYSCALSNNFARSFHPMLRYTNGP  
 PPL (SEQ ID NO:1079),  
 MKLLGECSSSIDSVKRLEHKLKEEEESLPGFVNLHSTETQTAGVIDRWELLQA  
 20 QALSKELRMKQNLQKWQQFNSDLNSIWAWLGDTEEELEQLQRLELSTDIQTI  
 ELQIK (SEQ ID NO:1080),  
 KLKELQKAVDHRKAILLSINLCSPEFTQADSKESRDLQDRLXQMNGRWDRVC  
 SLLEEWGRLLQDALMQCQGFHEMSHGLLLMLENIDRRKNEIVPIDSNLDAEIL  
 QDHHKQLMQIKHELLESQLRVASLQDMSCQL (SEQ ID NO:1081),  
 25 QDMSCQLLVNAEGTDCLEAKEKVHVIGNRLKLLLKEVSRHIKELEKLLDVSS  
 SQDLSSWSSADELDTSGSVSPXSGRSTPNRQKTPRGKCSLSQPGPSVSSPHS  
 (SEQ ID NO:1082),  
 DSSLSEXPGRSGRGFLFRVLRAALPLQLLLLLLIGLACLVPMSEEDYSCALSN  
 NFARSFHPMLRYTNGPPPL (SEQ ID NO:1083),  
 30 QRFLPPGSCXLIRGPQCPRVTDPTTGQSLDDSRFQIQQTENIIRSKTPTGPELDT  
 SYKGY (SEQ ID NO:1084),  
 SISASRLESIGTISFFLLSMFSSIRSKPWLISWKPWHCIRASCSRPRHSSSREHTR



SQRPFICXKRSCRSRLSLLSAWVNSGLQRLMERMALRWSTAFWSSLSFLIW  
SSMVWMSVLSSRRWSCSNSSSVSPSQAQMLFKSELNCCHFWRFCFILNSLLN  
AWAWRSSHRSTPAVWVSVLCRLTKPGRLLLLSSSLFTESILLHSPSSF  
M (SEQ ID NO:1085), TAFWSSLSFLIWSSMVWMSVLSSRRWSCSNSSSVS  
5 (SEQ ID NO:1086), LLNAWAWRSSHRSTPAVWVSVLCRL (SEQ ID NO:1087),  
LARHVLQRGYSELGFQQLMLYLHKL FVMVLKYLCIKVRINRDNFIFPSVNVL  
QHKKQTMAHFMETLALHQGILQQAPLLQQR AHSVPAPIHLXQAILQVPALL  
AVSLGELRAAEIDGEDDGFVVSFLELLELFDLELDGLDVSAEFQTLELFQL  
LLRVPQPGPDAVQV (SEQ ID NO:1088),

10 YSELGFQQLMLYLHKL FVMVLKYLCIKV (SEQ ID NO:1089),  
AMVCFLCWRTLTEGK (SEQ ID NO:1091), and/or  
VHSFLELLELFDLELDGLDVSAEFQTLEL (SEQ ID NO:1090). Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
15 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

20 This gene maps to chromosome 6, and therefore, may be used as a marker in  
linkage analysis for chromosome 6 (See Genbank Accession No. N62896).

This gene is expressed in numerous tissues including the heart, kidney, and  
brain.

Polynucleotides and polypeptides of the invention are useful as reagents for  
25 differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
musculoskeletal disorders including Muscular Dystrophy and cardiovascular diseases.  
Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
providing immunological probes for differential identification of the tissue(s) or cell  
30 type(s). For a number of disorders of the above tissues or cells, particularly of the  
muscle tissues, expression of this gene at significantly higher or lower levels may be  
routinely detected in certain tissues or cell types (e.g., muscle, heart, and cancerous

and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution in heart, combined with the homology to the human dystrophin gene indicates that the protein product of this gene is useful for the diagnosis and treatment of Muscular Dystrophy and other muscle disorders, particularly musculodegenerative conditions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets  
10       for the above listed tissues.

          Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:175 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
15       excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2365 of SEQ ID NO:175, b is an integer of 15 to 2379, where both a and b correspond to the positions of nucleotide  
20       residues shown in SEQ ID NO:175, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 166**

25       In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
GAGVGTAMPRVPQSAGGAVTWWGVGLSQPSSVQGGARPGTVPGTGPGPLPG  
LSPAPPPQHPPPLPKLFLCLSLXSLPQDFSLLLCLSLDPCPSSTSDL (SEQ ID  
NO:1092), GTVPGTGPGLPGLSPAPPPQHPPPLPKLFL (SEQ ID NO:1093),  
30       APSRCRRSVVQVPYSAFSSCSWTPTALRRGVLLYAGLSTSSASKAQGWHLG  
LEYPSGAIMEVRGRGGDRYAQGPSKCWRGCXLVGSGSVTAILCPGWGKAW  
DSARHPRTPSRLVSCSTASTPPTPAQAVSPLPLXFPAPGLLSSPLPLLGPLPFLY

L (SEQ ID NO:1094), TALRRGVLLYAGLSTSSASKAQGWHCLGLEYPGAIM (SEQ ID NO:1095), AILCPGWGKAWD SARHPRTPSRLVSCSTASTPP (SEQ ID NO:1096),

PPVFMASHRPXGMEPGEWRFVLVHIAFXCAWDLVCEHVSVC SQVRGRGRA

5 GVQGEAEEKREVLGQG XREAEEKQLGQGWGVLR RWSRRQAWKGSWGAW  
HCPRPCPTLDRGWL (SEQ ID NO:1097), and/or  
HVSVC SQVRGRGRAGVQGEAEEKREVLGQ (SEQ ID NO:1098). Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
10 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
polypeptides) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

15 This gene is expressed primarily in human cerebellum.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
diseases of the central nervous system, including Alzheimer's Disease, Parkinson's  
20 Disease, ALS, and mental illnesses. Similarly, polypeptides and antibodies directed  
to these polypeptides are useful in providing immunological probes for differential  
identification of the tissue(s) or cell type(s). For a number of disorders of the above  
tissues or cells, particularly of the central nervous system, expression of this gene at  
significantly higher or lower levels may be routinely detected in certain tissues or cell  
25 types (e.g., neural, and cancerous and wounded tissues) or bodily fluids (e.g., lymph,  
serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
taken from an individual having such a disorder, relative to the standard gene  
expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
individual not having the disorder.

30 Predicted epitopes include those comprising a sequence shown in SEQ ID  
NO: 414 as residues: Pro-20 to Gly-26, Leu-37 to Pro-42, His-57 to Gly-63.

"09236" 03204

The tissue distribution in human cerebellum indicates that the protein products of this gene are useful for the treatment/diagnosis of diseases of the central nervous system and may protect or enhance survival of neuronal cells by slowing progression of neurodegenerative diseases. Moreover, the protein product of this gene is useful

5 for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Protein, as well as, antibodies directed against

10 15 20 the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:176 and may have been publicly available prior to conception

25 of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1334 of SEQ ID NO:176, b is an

30 integer of 15 to 1348, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:176, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 167**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MKLLICGNYLAPSHSESSRRCCLLCFYPLCLEINFGMKVFLSMPFLVLFQSLIQ  
ED (SEQ ID NO:1099). Moreover, fragments and variants of this polypeptide (such  
as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridize, under stringent conditions, to the  
polynucleotide encoding this polypeptide are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the  
invention. Polynucleotides encoding this polypeptide are also encompassed by the  
invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
15. Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 15.

This gene is expressed primarily in human testes tumor, and to a lesser extent,  
in normal human testes.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
diseases of the testes, particularly cancer, and other reproductive disorders. Similarly,  
polypeptides and antibodies directed to these polypeptides are useful in providing  
immunological probes for differential identification of the tissue(s) or cell type(s).  
For a number of disorders of the above tissues or cells, particularly of the male  
reproductive tissues, expression of this gene at significantly higher or lower levels  
may be routinely detected in certain tissues or cell types (e.g., reproductive, testicular,  
and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, seminal  
fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
taken from an individual having such a disorder, relative to the standard gene

expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in human testicular tissue indicates that the protein products of this gene are useful for the treatment/diagnosis of reproductive diseases including cancers. Moreover, the protein may possibly have utility as a contraceptive or may be used to ameliorate disorders related to aberrant male secondary characteristics (e.g. hair, etc.). Protein, as well as, antibodies directed against the protein may, show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:177 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1488 of SEQ ID NO:177, b is an integer of 15 to 1502, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:177, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 168**

The translation product of this gene was found to have homology to the gar2 gene product of *Schizosaccharomyces pombe*, which is thought to be involved in protein metabolism (See Genbank Accession No. gi|663262).

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
 FSSPQGLKFRSKSSLANYLHKNGETSLKPEDFDFTVLSKRGIKSRYKDSCS (SEQ  
 ID NO:1100),  
 ELLCYICWKNTGLFSFFLSVFRGMVSSVKSFLVGEQLLSISEPRFKMSVCKCSF  
 LSTTSTFVPISSDSKKVSSYFSLCESLAEQNLFMMPEVFCSEQKFDPELNDLSF

FFTRLFSSLVTLRVSPHAPASEMQTVLS (SEQ ID NO:1101), and/or  
TFVPISSDSKKVSSYFSLCSESLAEQNLFMMPEVFC (SEQ ID NO:1102).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
5 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

10 The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

This gene is expressed primarily in fetal liver.

Polynucleotides and polypeptides of the invention are useful as reagents for  
15 differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatic disorders, in addition to conditions affecting hematopoietic development and metabolic diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
20 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hepatic system, and fetal hematopoietic system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, metabolic, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, bile, plasma,  
25 urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID  
30 NO: 416 as residues: His-7 to Trp-17, Leu-19 to Lys-27, Pro-33 to Gly-44, Lys-68 to Gly-74, Lys-85 to Cys-95.

T03220-292E560

The tissue distribution in liver, combined with the homology to the gar2 protein, indicates that the protein products of this gene are useful for the treatment/diagnosis of diseases of the developing liver and hematopoietic system, and act as a growth differentiation factor for hematopoietic stem cells. Moreover, the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition, the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders, and various would-healing models and/or tissue trauma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:178 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1623 of SEQ ID NO:178, b is an integer of 15 to 1637, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:178, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 169**

The polypeptide encoded by this gene is believed to be a membrane bound receptor.

Additionally, the extracellular domain of this polypeptide is expected to comprise the following amino acid sequence:

RILLVKYSANEENKYDYLPPTTVNVCSELVKLVFCVLVSFCVIKKDHQSRNLK  
YASWKEFSDFMKWSIPAFLYFLDNLIVFYVLSYLQPAMAVIFS NFSIITALLF



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a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 417 as residues: Met-1 to Cys-6, Ala-41 to Tyr-49, Lys-76 to Lys-84.

5           The tissue distribution in osteoclastoma and chondrosarcoma indicates that the protein products of this gene are useful for the diagnosis of cancers of the bone and connective tissues, and may act as growth factors for cells involved in bone or connective tissue growth. Moreover, this gene product may show utility in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis, bone cancer, as well as, disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis, as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e.

10           spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

15           

          Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:179 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

20           more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2897 of SEQ ID NO:179, b is an integer of 15 to 2911, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:179, and where b is greater than or equal to a + 14.

25           

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 170**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: NSVPNLQTLAVLTEAIGPEPAIPRXPREPPVATSTPATPSAGPQPLPTGTVLVPG GPAPPCLGEAWALLPPCRPSLTSCFWSPRPSPWKETGV (SEQ ID NO:1108),  
 5 VTAGRVGGGGPMPPQGKVGQDPQGPARSRLGGAGARQRVWQVWTWQ  
 QAAPGGXGGWRALGQWPQ (SEQ ID NO:1109),  
 STPATPSAGPQPLPTGTVLVPGGPAP (SEQ ID NO:1110), and/or  
 QDPQGPARSRLGGAGARQR (SEQ ID NO:1111). Moreover, fragments and  
 variants of these polypeptides (such as, for example, fragments as described herein,  
 10 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
 under stringent conditions, to the polynucleotide encoding these polypeptides) are  
 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 15 also encompassed by the invention.

This gene is expressed primarily in hematopoietic progenitor cells.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 20 hematopoietic or immune disorders, particularly cancer and autoimmune disorders.  
 Similarly, polypeptides and antibodies directed to these polypeptides are useful in  
 providing immunological probes for differential identification of the tissue(s) or cell  
 type(s). For a number of disorders of the above tissues or cells, particularly of the  
 blood/circulatory system, expression of this gene at significantly higher or lower  
 25 levels may be routinely detected in certain tissues or cell types (e.g., hematopoietic,  
 immune, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum,  
 plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken  
 from an individual having such a disorder, relative to the standard gene expression  
 level, i.e., the expression level in healthy tissue or bodily fluid from an individual not  
 30 having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID  
 NO: 418 as residues: Gln-4 to His-10, Pro-25 to His-32.

T02289-292660

The tissue distribution in hematopoietic progenitor cells indicates that the protein products of this gene are useful for diagnosis of diseases involving growth differentiation of hematopoietic cells. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as

5 anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such

10 as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed

15 tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:180 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 505 of SEQ ID NO:180, b is an integer of 15 to 519, where both a and b correspond to the positions of nucleotide

25 residues shown in SEQ ID NO:180, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 171**

30 In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 ALQLAFYPDAVEEWLEENVHPSLQRLQXLLQDLSEVSAPP (SEQ ID

NO:1112), CHPPALAGTLLRTPEGRAHARGLLLEAGGA (SEQ ID NO:1113),  
GSSSTRSWFSTSSPQRSASWHSGAPSCRSWRLPCSWLSTRMPWRSGWRKTCT  
PACSGCK (SEQ ID NO: 1114),

ASTLQPSLSPSSPPLXPPVETAVXSRALRREGAGSFPGSNILALVTQVSLHLRSS

5 VDALLEGNRYVTGWFSPTYHRQRKLIHPV (SEQ ID NO:1115),

PLGPEKAGLAXPLVXHAARPCPSTSLQSQCSPLXXEPXXPPRSXVISGGFDE

DVKAKVENLLGISSLEKTDVPRQAPCSPCPLLPLPFXRPWRQLFSAGLSAGR

GPAPSLAATSLPLSHKSASICAALWMRCWRATGMSLAGSAPTTASGSSSTRS

WFSTSSPQRSASWHSGAPSCRSWRLPCSWLSTRMPWRSGWRKTCTPACSGC

10 KLCCRTSARCLPPRCHPPALAGTLLRTPEGRAHARGLLLEAGGALXXXXAW

AIRPTWASCPAQCLAHTQFLRALGSPWGRD (SEQ ID NO:1116),

FQEDLMKMLKRKWRTFSGFPAWKKRTLLGKHPAALPVFFPSPSPARGDSCX

QQGSPQGGGRLLPWQQHPCPCHTSQPPSAQLCGCAAGGQQVCHWLQVPLPP

PAEAHPPGHGSAHPARSAQPPGTVEHPRAGAGGCPAAGFLPGCRGGVAGGK

15 RAPQAAAAAXSAAGPQRGVCPPAATHQPWQGRCSGPLRGELMPGGSCWRL

GGLCXXXWPGQYGPRGRRALWPSSVLPTLSS (SEQ ID NO:1117),

ALPSGVLSNVPARAGGWQRGGRHLAEVLQQSLQPLQAGVHVFLQPLLHGIR

VESQLQGSLLHEGAPLCQEAERCGLDVLNHDRVDELPLAVVGAEPASDIP

VALQQRIHRAAQMEADLCKGKDVAAREGAGPLPAESPAENSCLHGRXKGR

20 GRRGQGGGLQGACLTGSVFSRLEIPRRFSTFALTSSSNPPEITXXRGGXXGSXXR

EGLHWDCLVLVGHGRAAWXTNGQANPAFSGPKG (SEQ ID NO:1118),

RQLFSAGLSAGRGPAPSLAATSLPLSHKS (SEQ ID NO:1119),

ELPLAVVGAEPASDIPVALQQRIHRAAQ (SEQ ID NO:1120), and/or

QPPGTVEHPRAGAGGCPAAGFLPGCRG (SEQ ID NO:1121). Moreover,

25 fragments and variants of these polypeptides (such as, for example, fragments as

described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or

99% identical to these polypeptides and polypeptides encoded by the polynucleotide

which hybridizes, under stringent conditions, to the polynucleotide encoding these

polypeptides) are encompassed by the invention. Antibodies that bind polypeptides

30 of the invention are also encompassed by the invention. Polynucleotides encoding

these polypeptides are also encompassed by the invention.

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The protein product of this gene shares sequence homology with metallothionines. Thus, polypeptides encoded by this gene are expected to have metallothionine activity. Furthermore, such activities are known in the art and described elsewhere herein.

5 This gene is expressed primarily in kidney cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, renal disorders, particularly diseases of the kidney including cancer and renal  
10 dysfunction. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the renal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., renal,  
15 urogenital, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 419 as residues: Ser-47 to Gln-52.

The tissue distribution in kidney cortex indicates that the protein product of this gene is useful for the treatment/diagnosis of diseases of the kidney, including kidney failure. Moreover, this gene or gene product could be used in the treatment  
25 and/or detection of kidney diseases including nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Protein, as well as, antibodies  
30 directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

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Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:181 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 954 of SEQ ID NO:181, b is an integer of 15 to 968, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:181, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 172**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences: SVFERTNEFRDVLWSSI (SEQ ID NO:1122),

GVVQVTFMSSVSRVTWGCQPSICPGAPPAAALAGGLRLLFERELFGLPVSSPL  
ICSFLEHHPTSPPPSDCELLEGRSCVLLFIFLSPEPCTDPGMW (SEQ ID  
NO:1123),

SKQIHSFVHSFIHLFNTHLLSTYHIPGSVQSGDRKMNRRTQLLPSRSSQSDGG  
GDVLGWCSKKEQIRGEETGRPNSSLKRSRLRPPARAAAGGAPGQMLG (SEQ  
ID NO:1124), VTWGCQPSICPGAPPAAALAGGLRLLFE (SEQ ID NO:1125).  
and/or EQIRGEETGRPNSSLKRSRLRPP (SEQ ID NO:1126). Moreover, fragments

and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in 12 week old early stage human.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental abnormalities. Similarly, polypeptides and antibodies directed to these

5 polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developing embryo, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell

10 types (e.g., developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID

15 NO: 420 as residues: Gln-31 to Thr-43, Gly-51 to Ser-58, Pro-65 to Pro-72.

The tissue distribution in embryonic tissue indicates that the protein product of this gene is useful for treatment/diagnosis of developmental conditions. The gene may be involved in vital organ development in the early stage, especially hematopoiesis, the cardiovascular system, and neural development. Moreover,

20 expression within embryonic tissue indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again

25 be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

30 related to SEQ ID NO:182 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is



cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1114 of SEQ ID NO:182, b is an integer of 15 to 1128, where both a and b correspond to the positions of nucleotide  
 5 residues shown in SEQ ID NO:182, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 173

10 The translation product of this gene shares sequence homology with TGN38, an integral membrane protein previously shown to be predominantly localized to the trans-Golgi network (TGN) of cells. The gene encoding the disclosed cDNA is believed to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5.

15 This gene is expressed primarily in developing embryo, and to a lesser extent, in cancer tissues including lymphoma, endometrial, prostate and colon.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
 20 developmental abnormalities and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the developing fetus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or  
 25 cell types (e.g., developmental, reproductive, immune, gastrointestinal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an  
 30 individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 421 as residues: His-65 to Ser-72, Pro-82 to Gly-91, Pro-98 to Glu-118, Ser-126 to Gly-166, Pro-180 to Asp-188, Tyr-209 to Lys-214, Gln-220 to Leu-228.

The tissue distribution in the embryo, combined with the homology to an integral membrane protein indicates that the protein product of this gene is useful for the diagnosis of cancers and developmental abnormalities where aberrant expression relates to an abnormality. Expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:183 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2262 of SEQ ID NO:183, b is an integer of 15 to 2276, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:183, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 174**

The translation product of this gene shares sequence homology with a dnaJ heat shock protein from E. coli which is allelic to sec63, a gene that affects transit of nascent secretory proteins across the endoplasmic reticulum in yeast.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

QWEHLLLLPHLLRGAHRDPGDILPLAPRSECRANSIKEYQKSIWKVYVVRRLRL  
LKPQPNIIPTVKKIVLLAGWALFLFLAYKVSCTDREYQEYNPYEVLNLDPGAT

5 VAEIKKQYRLLSLKYHPDKGGDEV (SEQ ID NO:1127),  
EERGGGGGAMAGQQFQYDDSGNTFFYFLTSTFVGLVIVPATYYLWPRDQNAEQ  
IRLKNIRKVYGRG (SEQ ID NO:1128),

RLYTGCVIFDLVSNRALSFRMLCCNSCHSASSSLFCFSSCSLSESLSPSSFSL  
WESLLVSSSSSESLPLSETSSSSSFTAASFPTTPFACFCFCCFDCGNSTGVGFFFK  
10 GFFFFDLAVFLGPLLFCCHPPFVLFLLVSPCPSSAGCSSAAQMDCSFSNTSAIV  
CLVNLNTNTVTKDPTVMLLLSSSSSNTCDFISMVTYGKLPRTAITSSYFSSSRKCS  
RV (SEQ ID NO:1129), YQKSIWKVYVVRRLRLKPQPNIIPTVKKIVLLAGW  
(SEQ ID NO:1130), and/or CHPPFVLFLLVSPCPSSAGCSSAAQMDCSFSNTSA  
(SEQ ID NO:1131). Moreover, fragments and variants of these polypeptides (such

15 as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%,  
95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
polynucleotide encoding these polypeptides) are encompassed by the invention.  
Antibodies that bind polypeptides of the invention are also encompassed by the  
20 invention. Polynucleotides encoding these polypeptides are also encompassed by the  
invention.

This gene is expressed primarily in Hodgkin's lymphoma, and to a lesser extent, in testes.

Polynucleotides and polypeptides of the invention are useful as reagents for  
25 differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
immune or hematopoietic disorders, particularly cancer. Similarly, polypeptides and  
antibodies directed to these polypeptides are useful in providing immunological  
probes for differential identification of the tissue(s) or cell type(s). For a number of  
30 disorders of the above tissues or cells, particularly of the immune system, expression  
of this gene at significantly higher or lower levels may be routinely detected in certain  
tissues or cell types (e.g., immune, hematopoietic, reproductive, testicular, and

cancerous and wounded tissues) or bodily fluids (e.g., lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 422 as residues: Val-37 to Pro-49, His-76 to Asp-82, Thr-97 to Trp-105, Arg-158 to Asp-165, Glu-199 to Asp-214, Asn-229 to Pro-236, Thr-261 to Gln-266, Arg-292 to Glu-298, Glu-335 to Lys-351, Glu-372 to Glu-377, Leu-398 to Asn-405, Glu-437 to Pro-480, Gln-487 to Gln-495, Lys-507 to Ala-555, Ser-563 to Arg-569, Pro-588 to Glu-593, Lys-618 to Val-623, Pro-630 to Asn-635, Ser-644 to Gly-649, Lys-664 to Trp-673, Gly-679 to Phe-689, Asp-691 to Asp-704.

The tissue distribution in Hodgkin's lymphoma, combined with the homology to dnaJ and sec63 indicates that the protein product of this gene is useful as a diagnostic for cancer, that the protein may be useful in regulating gene expression levels, and that it is essential for normal protein metabolism. Therefore, protein products of this gene may show utility as an anticancer agent, or even serve to protect from viral or bacterial infections, based upon its homologous function as a protein chaperone. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:184 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3360 of SEQ ID NO:184, b is an integer of 15 to 3374, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:184, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 175

The gene encoding the disclosed cDNA is believed to reside on chromosome

5. Accordingly, polynucleotides related to this invention are useful as a marker in  
 5 linkage analysis for chromosome 5. Contact of cells with supernatant expressing the  
 product of this gene has been shown to increase the permeability of the plasma  
 membrane of chondrocytes to calcium. Thus it is likely that the product of this gene  
 is involved in a signal transduction pathway that is initiated when the product binds a  
 receptor on the surface of the plasma membrane of both chondrocytes, in addition to  
 10 other cell-lines or tissue cell types. Thus, polynucleotides and polypeptides have uses  
 which include, but are not limited to, activating chondrocytes.

This gene is expressed primarily in endothelial cells, and to a lesser extent, in  
 bone marrow stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 15 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 and for diagnosis of diseases and conditions which include, but are not limited to,  
 immune, hematopoietic, endothelial, or vascular disorders, such as diseases involving  
 angiogenic abnormalities including diabetic retinopathy, macular degeneration, and  
 other diseases including arteriosclerosis and cancer. Similarly, polypeptides and  
 20 antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of  
 disorders of the above tissues or cells, particularly of the vascular system, expression  
 of this gene at significantly higher or lower levels may be routinely detected in certain  
 tissues or cell types (e.g., immune, hematopoietic, endothelial, vascular, and  
 25 cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine,  
 synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
 individual having such a disorder, relative to the standard gene expression level, i.e.,  
 the expression level in healthy tissue or bodily fluid from an individual not having the  
 disorder.

30 The tissue distribution in endothelial cells indicates that the protein products  
 of this gene are useful for treating diseases where an increase or decrease in  
 angiogenesis is indicated and as a factor in the wound healing process. In addition,

the protein product of this gene may show utility in the treatment, detection, and/or prevention of a variety of vascular disorders, which include, but are not limited to microvascular disease, embolism, thrombosis, atherosclerosis, aneurysm, or stroke. Moreover, the protein product of this gene is useful for the treatment and diagnosis of

5 hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in

10 lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as

15 a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:185 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

20 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1323 of SEQ ID NO:185, b is an integer of 15 to 1337, where both a and b correspond to the positions of nucleotide

25 residues shown in SEQ ID NO:185, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 176**

30 The translation product of this gene shares sequence homology with both the RIC and MAT8 proteins (mouse), which are thought to be important in regulating

chloride conductance in cells by modulating the response mediated by cAMP and protein kinase C to extracellular signals.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

- 5 GTSLDAAATAASLSPRGCRRLRTPSSD (SEQ ID NO:1132),  
 QIQRHTRAPKQLIPLMTPRRSLRDHPQAQTSRQTPRPSSHLVFMRMTPSSMM  
 NTPSGNGGCWSQLCCSSQASSSSPVASAGSCPGYAGIAGESIRNRS (SEQ ID  
 NO:1133), PRRSLRDHPQAQTSRQTPRPSSHLVFM (SEQ ID NO:1134),  
 THPPETGAVGRSCAVHHRHHHPHQWQVQAAVPVMPESLQVSPSETGADNXL  
 10 GTRRPSPLPAHRAQPPASPRRAWPEREDTDDEAGARAAGPSLLPPPTLPAPEG  
 YLAPWGLSLKLSPLLRLQKVKHCGLC (SEQ ID NO:1135),  
 PESLQVSPSETGADNXLGTRRPSPLPAHRAQPPASP (SEQ ID NO:1136),  
 GTAPKAPGSLQGRAGLGEVGDSRQPWLQLHHLCLPSLARLFEGMQEAGHG  
 ELAGGLVFGCPAGCQLFLMDSPAMIPA (SEQ ID NO:1137),  
 15 GEVGDSRQPWLQLHHLCLPSLARLFEGMQEAGH (SEQ ID NO:1138),  
 GSGGLSGRLCLGMVSQRASWCHQWDELLWCSCVSLDLSLEAHPFLPVAGSG  
 SGVVVFHQQARLGLERWAGVLCRLHLGLVSGPECP (SEQ ID NO:1139),  
 and/or QWDELLWCSCVSLDLSLEAHPFLPVAGSGSGVVVFHQQARL (SEQ ID  
 NO:1140). Moreover, fragments and variants of these polypeptides (such as, for  
 20 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides) are encompassed by the invention. Antibodies that bind  
 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
 25 encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 19. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 19.

- This gene is expressed primarily in amniotic cells and hematopoietic cells  
 30 including macrophages, neutrophils, T cells, TNF induced aortic endothelium, and to  
 a lesser extent in testes, TNF induced epithelial cells, and smooth muscle.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune or hematopoietic disorders, particularly inflammatory responses mediated by

5 T cells, macrophages, and/or neutrophils, particularly those involving TNF, and also cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or

10 lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from

15 an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 424 as residues: Thr-19 to Ala-33, Leu-54 to Asp-82, Pro-89 to Ala-97, Pro-100 to Lys-125, Ser-127 to Phe-135, Gly-164 to Leu-169, Cys-173 to Arg-178.

The tissue distribution in hematopoietic cells, combined with the homology to

20 the RIC and mat-8 genes, indicates that the protein product of this gene is useful for modifying inflammatory responses to cytokines such as TNF, and thus modifying the duration and/or severity of inflammation. Polynucleotides and polypeptides derived from this gene are thought to be useful in the diagnosis and treatment of cancer. The protein product of this gene is useful for the treatment and diagnosis of hematopoietic

25 related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can

30 be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and



in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:186 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 927 of SEQ ID NO:186, b is an integer of 15 to 941, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:186, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 177**

This gene is expressed primarily in endothelial cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, vascular disorders, including vascular restenosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., vascular, endothelial, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in endothelial tissue indicates that the protein product of this gene is useful for treating diseases associated with vascular responses to injury such as vascular restenosis following angioplasty. Moreover, the protein product of this gene is useful for the treatment, detection, and/or prevention of a variety of other vascular disorders, which include, but are not limited to microvascular disease, embolism, thrombosis, atherosclerosis, aneurysm, or stroke. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:187 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 664 of SEQ ID NO:187, b is an integer of 15 to 678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:187, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 178**

This gene appears to be chimeric. There are two ORFs of interest. The first ORF-1 encodes a polypeptide preferably comprising one of the following polypeptide sequences:

MRPDWKAGAGPGGPPQKPAPSSQRKPPARPSAAAAIAVAAEEERRLRQR  
 NRLRLEEDKPAVERCLEELVFGDVENEDALLRRLRGPRVQEHEDSGDSEVE  
 NEAKGNFPPQKKPVWVEEDEDEEMVDMNNRFRKDMMKNAESKLSKD  
 NLKKRLKEEFQHAMGGVPAWAETTKRKTSSDDESEDEDDLLQRTGNFISTS  
 TSLPRGILKMKNQCQHANAERPTVARISICAVPSRCTDCDGCWD (SEQ ID  
 NO:1141); and/or  
 CLEELVFGDVENEDALLRRLRGPRVQEHEDSGDSEVENEAKGNFPPQKKPV

WVDEEDEDEEMVDMMNRRFRKDMMKNAESKLSKDNLKKRLKEEFQHAM  
GGVPAWAETTKRKTSSDDESEDEDDLLQRTGNFISTSTSLPRGILKMKNQCQ  
ANAERPTVARISICAVPSRCTDCDGC (SEQ ID NO:1142). The second ORF  
(ORF-2) encodes a polypeptide preferably comprising one of the following

5 polypeptide sequences:

LKEKIVRSFEVSPDGSFLLINGIAGYLHLLAMKTKELIGSMKINGRVAASTFSS  
DSKKVYASSGDGEVYVWDVNSRKCLNRFVDEGSLYGLSIATSRNGQYVACG  
SNCGVVNIYNQDSCLQETNPKPIKAIMNLVTGVTSLTFNPTTEILAIASEKMKE  
AVRLVHLPSTVFSNFPVIKKNKNISHVHTMDFSPRSGYFALGNEKGKALMYR

10 LHHYSDF (SEQ ID NO:1143); and/or

KINGRVAASTFSSDSKKVYASSGDGEVYVWDVNSRKCLNRFVDEGSLYGLSI  
ATSRNGQYVACGSNCGVVNIYNQDSCLQETNPKPIKAIMNLVTGVTSLTFNP  
TTEILAIASEKMKEAVRLVHLPSTVFSNFPVIKKNKNISHVHTMDFSPRSGYFA  
LGNEKGKAL (SEQ ID NO:1144).

15 In specific embodiments, polypeptides of the invention comprise, or alternatively  
consist of, the following amino acid sequences:

WLLGLDNAVSLFQVDGKTNPKIQSIYLERFPIFKACFSANGEEVLATSTHSKV  
LYVYD (SEQ ID NO:1145), LVFGDVENDEEDALLRRLRGPRVQ (SEQ ID  
NO:1146), KNAESKLSKDNLKKRLKEEFQHAMGGVP (SEQ ID NO:1147),

20 and/or SLPRGILKMKNQCQHANAERPTVA (SEQ ID NO:1148). Moreover,  
fragments and variants of these polypeptides (such as, for example, fragments as  
described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
which hybridizes, under stringent conditions, to the polynucleotide encoding these  
25 polypeptides) are encompassed by the invention. Antibodies that bind polypeptides  
of the invention are also encompassed by the invention. Polynucleotides encoding  
these polypeptides are also encompassed by the invention.

The translation product of this gene shares homology with the transcriptional  
repressor TUP1 of *Candida albicans* (See Genbank Accession No. gi|2245634  
30 (AF005741)), which is thought to modulate the expression levels of cellular filament  
and may implicate this protein as serving a useful role in the amelioration of  
proliferating cells and tissues.

This gene is expressed primarily in epididymus and endometrial tumors, and to a lesser extent, in T cell lymphoma and cell lines derived from colon cancer.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive or developmental conditions, which include tumors of the reproductive organs, including testis and endometrial cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 426 as residues: Ser-67 to Lys-72, Val-87 to Leu-93, Tyr-128 to Pro-141, Asp-204 to Gly-210.

The tissue distribution in reproductive tissue cancers, combined with the homology to a transcriptional repressor protein, indicates that the protein products of this gene are useful for treating tumors of the endometrium or epithelial tumors of the reproductive system. Moreover, the protein may also be useful as a contraceptive. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:188 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or

more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1834 of SEQ ID NO:188, b is an integer of 15 to 1848, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:188, and where b is greater than or equal to a + 14.

5

## FEATURES OF PROTEIN ENCODED BY GENE NO: 179

In specific embodiments, polypeptides of the invention comprise, or

10 alternatively consists of, an amino acid sequence selected from the group:

MRILQLILLALATGLVGGETRIIKGFECKLHSQPWQAALFEKTRLLCGATLIAP  
RWLLTAAHCLKPRYIVHLGQHNLQKEEGCEQTRTATESFPHPGFNNSLPNKD  
HRNDIMLVKMASPV SITWAVRPLTLSSRCVTAGTSCSFPAGAARPDPSYACLT  
PCDAPTSPSLSTRSVRTPTPATSQTPWCVPACRKGARTPARVTPGALWSVTSL

15 FKALSPGARIRVRSPESLVSTRKSANMWTGSRRR (SEQ ID NO:1149);

ETRIIKGFECKLHSQPWQAALFEKTRLLCGATLIAPRWLLTAAHCLKPRYIVH  
LGQHNLQKEEGCEQTRTATESFPHPGFNNSLPNKDHRNDIMLVKMASPV SIT  
WAVRPLTLSSRCVTAGTSCSFPAGAARPDPSYACLT PCDAPTSPSLSTRSVRTP  
TPATSQTPWCVPACRKGARTPARVTPGALWSVTSLFKALSPGARIRVRSPESL

20 VSTRKSANMWTGSRRR (SEQ ID NO:1150); and/or

CKLHSQPWQAALFEKTRLLCGATLIAPRWLLTAAHCLKPRYIVHLGQHNLQK  
EEGCEQTRTATESFPHPGFNNS (SEQ ID NO:1151). Moreover, fragments and

variants of these polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to

25 these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides) are  
encompassed by the invention. Antibodies that bind polypeptides of the invention are  
also encompassed by the invention. Polynucleotides encoding these polypeptides are  
also encompassed by the invention.

30 The translation product of this gene shares sequence homology with  
neuropsin, a novel serine protease, which is thought to be important in modulating  
extracellular signaling pathways in the brain. Owing to the structural similarity to

other serine proteases, the protein products of this gene are expected to have serine protease activity which may be assayed by methods known in the art and described elsewhere herein. Moreover, this protein has been shown to also have homology to PSA (prostate specific antigen). PSA is a serum marker for prostate cancer and it is a member of the kallikrein family. The members of the kallikrein family are secreted serine proteases and some of them are good tissue specific markers. This new member of the kallikrein family has been detected twice in endometrial tumor cDNA library and therefore is a good candidate as a serum marker for endometrial tumor.

This gene is expressed primarily in endometrial tumor, and to a lesser extent, in colon cancer, benign hypertrophic prostate, and thymus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive, immune, or endocrine disorders, particularly cancers of the endometrium or colon and benign hypertrophy of the prostate. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the urogenital or reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, immune, endocrine, gastrointestinal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 427 as residues: Glu-27 to Trp-35, Leu-77 to Ala-89, Pro-96 to Asn-109, Ser-149 to Arg-156, Gln-172 to Ile-182, Glu-193 to Gly-204, Glu-245 to Asn-250.

The tissue distribution in proliferative reproductive tissues, combined with the homology to serine proteases indicates that the protein product of this gene is useful for diagnosing, treating, and/or preventing hyperproliferative disorders such as cancer of the endometrium or colon and hyperplasia of the prostate. Similarly, expression

within cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:189 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1278 of SEQ ID NO:189, b is an integer of 15 to 1292, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:189, and where b is greater than or equal to a + 14.

#### *FEATURES OF PROTEIN ENCODED BY GENE NO: 180*

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: VLQGRYFSPILEMRRLRPEGXXNLPGGSRAQKEPRQDLTLVLWPHCPHFAMT RSYVPTKQCMVQGSFYCIFIKGPVQNWC (SEQ ID NO:1152), and/or CPRRRTCVRVEKSRPFQCQLHSIS (SEQ ID NO:1153). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are

also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal brain.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neural disorders, particularly neurodegenerative conditions, in addition to identifying and expanding stem cells in the CNS. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 428 as residues: Met-1 to Lys-9, Glu-26 to Lys-37, Lys-39 to Lys-48.

The tissue distribution in fetal brain indicates that the protein products of this gene are useful for detecting and expanding stem cell populations in the (or of the) central nervous system. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene



product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:190 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 892 of SEQ ID NO:190, b is an integer of 15 to 906, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:190, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 181**

In specific embodiments, polypeptides of the invention comprise, or alternatively  
20 consist of, the following amino acid sequences: PKEPGVPE (SEQ ID NO:1154), LQLKPRDPFSTLGPNAVLSPQRLVLETLSKLSIQDNNVDLILATPPFSRLEKLY STMVRFLSDRKNPVCRRWLWYCWPTWLRGTAWQLVPLQCRRRAVSATSWAS (SEQ ID NO:1155), RDPFSTLGPNAVLSPQRLVLETLSKLS (SEQ ID NO:1156), EVISGLFIQSRRRERGQGVVGSHMILWGKSLFFFSPQRLTKNIFKNYSLLLTQR  
25 FLFPCETLLQYVYSIRCTVQYMKGSTLYCTGLSSEQGLFTTANFLAPARL (SEQ ID NO:1157), and/or IRCTVQYMKGSTLYCTGLSSEQG (SEQ ID NO:1158). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
30 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind

This gene is expressed primarily in early stage human brain, fetal liver/spleen, and stromal cells.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental abnormalities, neural, immune, or hematopoietic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s).

For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., developmental, neural, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEO ID

20 NO: 429 as residues: Gln-42 to Gln-47, Gln-54 to Pro-60.

The tissue distribution in embryonic brain and fetal liver indicates that the protein products of this gene play a role in the development of the central nervous and hematopoietic systems. Therefore this gene and its products are useful for diagnosing or treating developmental abnormalities of the central nervous system. Moreover, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in

the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:191 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1927 of SEQ ID NO:191, b is an integer of 15 to 1941, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:191, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 182**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: MPIIDQVNPELHDFMQSAEVTIFALSWLITWFGHVLSDFRHVVRLYDFFLAC HPLMPIYFAAVIVLYREQEVLDCDCDMASVHHLLSQIPQDLPYETLISRSETFL FSPHPNLLGRPLPNSKLRGRQPLLSKTLSTWHQPSRGLIWCCGSGXRGLLRPE DRTKDVLTTPRTNRFVKLAVMGLTVALGAAALAVVKSALWAPKFQLQLFP (SEQ ID NO:1159; "ORF-1"); or CPEFFIPATLPCPFVFAFTSEASSRAYLTQRGPGGLAQNLMPVGFWMGSLP PPWCWRKWVSEACSCFC (SEQ ID NO:1160; "ORF-2"). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides

of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

ORF-2 is structurally similar to various TGF-beta family members. Thus, this polypeptide is expected to have a variety of activities in the modulation of cell growth and proliferation.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

CRQAGAVRGHPMFQFTFYGVTXRFPVTRAAQAQQVAKAAASFRNPLPPTPG  
RWQRAHPKAHWERHKILCQAPRSPLCQVGSATGL (SEQ ID NO:1161),

HILNYLMPIDQVNPELHDFMQSAEVTIFALSWLITWFGHVLSDFRHVVRLY  
DFFLACHPLMPIYFAAVIVLYREQEVLDCDCDMASVHHLLSQIPQDLPYETLIS  
RXETFLFSFHPNLLGRPLPNSKLRGRQPLLSTLSWHQPSRGLIWCCGSGXR  
GLLRPEDRTKDVLTKPRTNRFVKLAVMGLTVALGAAALAVVKSALWAPKF  
QLQLFP (SEQ ID NO:1162), AEGVTIFALSWLITWFGHVLSDFRHVVRLYD

(SEQ ID NO:1163), and/or VLTKPRTNRFVKLAVMGLTVALGAAALAVVKS  
(SEQ ID NO:1164). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 20. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 20.

This gene is expressed primarily in osteoclastoma, microvascular endothelium, and bone marrow derived cell lines.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, skeletal, vascular, or hematological diseases, particularly those involving aberrant

proliferation of stem cells. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at

5 significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skeletal, vascular, immune, hematological, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in

10 healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 430 as residues: Ser-33 to Ala-39.

The tissue distribution in bone marrow and endothelial cells indicates that the protein products of this gene is useful for treating disorders of the progenitors of the

15 immune system. Applications include in vivo expansion of progenitor cells, ex vivo expansion of progenitor cells, or the treatment of tumors of the circulatory system, such as lymphomas. Moreover, the protein product of this gene may also show utility in either the enhancement or inhibition of immune cell localization or targeting at sites of inflammation or injury. The protein product of this gene may be useful in the

20 treatment, detection, and/or prevention of a variety of vascular disorders, which include, but are not limited to microvascular disease, embolism, aneurysm, atherosclerosis, or stroke. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:192 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

30 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2104 of SEQ ID NO:192, b is an

integer of 15 to 2118, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:192, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 183

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: GFGSVSAAGRSGGTWQPVQ (SEQ ID NO:1165), PGGLAVG SRW WSRSLT (SEQ ID NO:1166), LEPSRQRRPRRRGGTSRPETDQRAKCWRQL (SEQ ID NO:1167), VCLRCQNRMEN (SEQ ID NO:1168), MAACTARRPGRGQPLVVPVADXGPVAKAALCAAXAGAFSPASTTTTRRHLS SRNRPEGKVLETVGVEVPKQNGKYETGQLFLHSIFGYRGVVLPWQARLXD RDVASAAPEKAENPAGHGSKEVKGKTHTYQVLIDARDCPHISQRSQTEAVT FLANHDDSRALYAIPGLDYVSHEDILPYTSTDQVPIQHELPERFLLYDQTKAPP FVARETLRAWQEKHPWLELSDVHRETTENIRVTVIPFYMGMRQAQNSHVY WWRYCIRLENLSDVQQLRERHWRIFSLSGTLETVRGRGVVGREPVLSKEQP AFQYSSHVSLQASSGHMWGTFRFERPDGSHFDVRIPPFSLSENKDEKTPPSGL HW (SEQ ID NO:1169), MAACTARRPGRGQPLVVPVADXGPVAKAALCAA (SEQ ID NO:1170), MAACTARRPGRGQPLVVPVADXGPVAKAALCAA (SEQ ID NO:1171), MAACTARRPGRGQPLVVPVADXGPVAKAALCAA (SEQ ID NO:1172), MAACTARRPGRGQPLVVPVADXGPVAKAALCAA (SEQ ID NO:1173), MAACTARRPGRGQPLVVPVADXGPVAKAALCAA (SEQ ID NO:1174), VLETVGVEVPKQNGKYETGQLFLHSIFGYRGVV (SEQ ID NO:1175), GLDYVSHEDILPYTST (SEQ ID NO:1176), DVHRETTENIRVTVIPFYM (SEQ ID NO:1177), WWRYCIRLENLSDVQQLRER (SEQ ID NO:1178), PAFQYSSHVSLQASSGHMWGTFRFER (SEQ ID NO:1179), RLPCHKRRCFCLVIQKKSFKFEMLDGNLISGGVGEDVFMADIVQAWDGIEGP TVIMVSQEGHSFCLRLRYMWAVTSINQHLIVSVSFAFHLLGAMASRVLCFF WSCRSHIPVXQSGLPKGQDDTSVAKNAMKEKLPGLIFSILFWHLKHTNCLQH FALWSVSGREVPPRRRGRRWREGSSXGRAQSGLGHRAXVSDRDHQRLPTAR

PPGCTGCHVPPERRPAADTEPNP (SEQ ID NO:1180),  
 KEFMLDGNLISGGVGEDVFMADIVQAWDGIE (SEQ ID NO:1181),  
 AVTSINQHLIVSVSFAFHLLGAMASRVLC (SEQ ID NO:1182), and/or  
 TARPPGCTGCHVPPERRPAA (SEQ ID NO:1183). Moreover, fragments and

5 variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 10 also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

15 This gene is expressed primarily in gall bladder, prostate, and fetal brain, and to a lesser extent, in tumor and fetal tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,  
 20 gastrointestinal, reproductive, neural, or growth related disorders such as cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate, gall bladder, and fetal brain, expression of this gene at significantly higher or  
 25 lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, reproductive, neural, developmental, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,  
 30 the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

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The tissue distribution in fetal brain and tumor tissues indicates that the protein product of this gene is useful for the diagnosis and treatment of growth-related disorders, such as cancers. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival, in addition to metabolic, or reproductive disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:193 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1524 of SEQ ID NO:193, b is an integer of 15 to 1538, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:193, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 184



In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: SLCCPEGAEGC (SEQ ID NO:1184), QLKKTHYDRPCP (SEQ ID NO:1185), QLKKTHYDRPCP (SEQ ID NO:1186),  
 5 MNRPCPFCLWKVFPLLLLLHEELFPLPVP (SEQ ID NO:1187), and/or KEKTFTPRNSLCCPEGAEGCIAGGDLQLKKTHY (SEQ ID NO:1188). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
 10 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in stromal cell, tonsil, and glioblastoma.

15 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hematopoietic, immune and inflammatory disorders, in addition to neural disorders, such as glioblastoma. Similarly, polypeptides and antibodies directed to these  
 20 polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the stromal cells, tonsil, and glioblastoma expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, neural, and cancerous and  
 25 wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Additionally, it is believed that the product of this gene regulates pancreatic cell  
 30 differentiation into beta cells. Accordingly, polynucleotides and polypeptides of the invention are useful in the treatment of insulin-dependent diabetes mellitus and

associated conditions e.g. pancreatic hypofunction and the prevention, as well as the treatment of undifferentiated type pancreatic cancers.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 432 as residues: Pro-27 to Ala-32.

5 The tissue distribution in stromal cells and tonsils indicates that the protein product of this gene is useful for diagnosis and treatment of immune and inflammatory disorders and glioblastoma. Similarly, the protein product of this gene is useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells  
10 are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene  
15 product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:194 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
25 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1084 of SEQ ID NO:194, b is an integer of 15 to 1098, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:194, and where b is greater than or equal to a + 14.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 185**

This gene is expressed primarily in hepatocellular carcinoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatic or metabolic diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., hepatic, metabolic, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, bile, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 433 as residues: Gly-32 to Lys-39.

The tissue distribution in hepatocellular carcinoma tissue indicates that the protein product of this gene is useful for diagnosis and treatment of liver diseases. Moreover, the protein product of this gene is useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). In addition the protein may have a useful role in treating, detecting, or preventing developmental abnormalities, fetal deficiencies, pre-natal disorders and various would-healing models and/or tissue trauma. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:195 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 987 of SEQ ID NO:195, b is an integer of 15 to 1001, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:195, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 186**

10 This gene is expressed primarily in hippocampus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neuronal or endocrine disorders, particularly behavioral and mood disorders.

15 Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hippocampus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, endocrine, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 434 as residues: Ser-14 to Tyr-20.

The tissue distribution in hippocampus indicates that the protein product of this gene is useful for the diagnosis and treatment of neuronal disorders. Moreover, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions which include, but are not limited to Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, meningitis, encephalitis, demyelinating

diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:196 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1444 of SEQ ID NO:196, b is an integer of 15 to 1458, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:196, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 187**

This gene is expressed primarily in bone cancer and hippocampus, and to a lesser extent, in osteoclastoma.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, bone-related disorders and neuronal diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for

differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the bone, osteoclast, and hippocampus, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, skeletal, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in hippocampus and skeletal tissues indicates that the protein product of this gene is useful for diagnosis and treatment of bone-related disorders and neuronal diseases. Similarly, this gene product is useful in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis, bone cancer, as well as, disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Alternatively, the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:197 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1268 of SEQ ID NO:197, b is an

integer of 15 to 1282, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:197, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 188

The gene encoding the disclosed cDNA is thought to reside on chromosome 4. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 4.

10 This gene is expressed primarily in neuronal tissues such as hippocampus, spinal cord, and hypothalamus.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, 15 neuronal diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neuronal tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell 20 types (e.g. neuronal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

25 The tissue distribution in neuronal tissues indicates that the protein product of this gene is useful for diagnosis and treatment of neuronal disorders, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, 30 including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked

disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:198 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 937 of SEQ ID NO:198, b is an integer of 15 to 951, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:198, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 189**

The gene encoding the disclosed cDNA is thought to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in neuronal tissues and immune tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neuronal and immune-related disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neuronal and immune-related tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. neuronal, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such



a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 437 as residues: Pro-19 to Asp-25.

5           The tissue distribution neuronal and immune tissues indicates that the protein product of this gene is useful for the diagnosis and treatment of neuronal and immune-related disorders. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the detection/treatment of neurodegenerative disease states, neuronal disorders, and behavioral disorders such as Alzheimer's

10   Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of

15   developmental disorders associated with the developing embryo, or sexually-linked disorders. Additionally, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as,

20   antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial

25   utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

30           Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:199 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1726 of SEQ ID NO:199, b is an integer of 15 to 1740, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:199, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 190

The translation product of this gene shares sequence homology with human N33, a gene located in a homozygously deleted region of human metastatic prostate cancer, which is thought to be important in prevention of prostate cancer. The gene and its translation product also share sequence homology with an isolated prostate/colon tumor suppressor gene (PSTG) product (WO9532214-A1.).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

AQRKKEMVLSEKVSQLEWTKRPMNGDKFRRLVKAPPRNYSVIVMFT  
ALQLHRQCVVCKQADEEFQILANSWRYSSAFTNRIFAMVDFDEGSDVFQML  
NMNSAPTFINFPAGKPKRGDTYELQVRGFSAEQIARWIADRTDVNIRVIRPP  
NMAARWRFWCVSVT (SEQ ID NO:1189), MVVALLIVCDVPSAS (SEQ ID  
NO:1190), AQRKKEMVLSEKVSQLEWTKRPMNGDKFRRLVKAPPRNYSVIVMFT  
ALQLHRQCVVCKQADEEFQILANSWRYSSAFTNRIFAMVDFDEGSDVFQML  
NMNSAPTFINFPAGKPKRGDTYELQVRGFSAEQIARWIADRTDVNIRVIRPPN  
NMAARWRFWCVSVT (SEQ ID NO:1193), MVDFDEGSDVFQMLNMNSAPTFINFPAGKPK  
RGDTYELQVRGFSAEQIARWIADRTDVNIRVIRPPN (SEQ ID  
NO:1194), KRGDTYELQVRGFSAEQIARWIADRTDVNIRVIRPPN (SEQ ID  
NO:1195), and/or  
YAGPLMLGLLAVIGGLVYLRRVIWNFSLIKLDGLLQLCVLCLL (SEQ ID  
NO:1196). Moreover, fragments and variants of these polypeptides (such as, for  
example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in infant adrenal gland, prostate cell line, and to a lesser extent in adrenal gland.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate cancer and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the prostate and adrenal gland, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. prostate, endocrine, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 438 as residues: Pro-34 to Gly-43, Arg-113 to Pro-120.

The tissue distribution infant adrenal gland, combined with the homology to N33 and prostate/colon tumor suppressor gene (PSTG) indicates that the protein product of this gene is useful for the diagnosis and treatment for prostate cancer and endocrine disorders, and that the nucleic acids and proteins of this gene can be used in the diagnosis and treatment of prostate, endocrine and colorectal cancers. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:200 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1693 of SEQ ID NO:200, b is an integer of 15 to 1707, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:200, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 191**

This gene is expressed primarily in T-cell, and to a lesser extent in fetal lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and respiratory disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and respiratory systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, respiratory, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 439 as residues: Trp-3 to Phe-9.

The tissue distribution in T-cells and fetal lung indicates that the protein product of this gene is useful for the diagnosis and treatment of immune and respiratory disorders. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor

marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Expression of this gene product in T cells also strongly indicates a role for this protein in immune function and immune surveillance. The tissue distribution also indicates that the protein product of this gene is useful for the detection and treatment of disorders associated with developing lungs, particularly in premature infants where the lungs are the last tissues to develop. The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and intervention of lung tumors, since the gene may be involved in the regulation of cell division, particularly since it is expressed in fetal tissue. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:201 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 765 of SEQ ID NO:201, b is an integer of 15 to 779, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:201, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 192**

The gene encoding the disclosed cDNA is thought to reside on chromosome 6. Accordingly, polynucleotides related to this invention are useful as a marker in

linkage analysis for chromosome 6. The translation product of this gene shares significant homology with the rat protein Neuritin, and in fact appears to be a human ortholog of the rat protein. It is believed that this gene is induced in rats by neural activity and neurotrophins, and that it promotes neuritogenesis. Neural activity and neurotrophins induce synaptic remodeling in part by altering gene expression. This gene is believed to be a glycosylphosphatidylinositol-anchored protein encoded by a hippocampal gene, and to possess neural activity. This molecule is believed to be expressed in post-mitotic differentiating neurons of the developing nervous system and neuronal structures associated with plasticity in the adult. Message of this gene is believed to be induced by neuronal activity and by the activity-regulated neurotrophins BDNF and NT-3. The product of this gene is believed to stimulate neurite outgrowth and arborization in primary embryonic hippocampal and cortical cultures, and to act as a downstream effector of activity-induced neurite outgrowth. In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: DAVFKGFSDCLLKLGD (SEQ ID NO:1197), CQEGAKDMWDKLRKESKNLN (SEQ ID NO:1198), VLLVSLSAALATWLSF (SEQ ID NO:1199), MGLKLNGRYISLILAVQIAYLVQAVRAAGKCD (SEQ ID NO:1200), PAAWDDKTNIKTVCTYWEDFHSCVTALTDCQEGAKDMWDKLRKESKNLN (SEQ ID NO:1201), and/or MGLKLNGRYISLILAVQIAYLVQAVRAAGKCD (SEQ ID NO:1202). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human placenta, endometrial tumor and tissues of the central nervous system (CNS).

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, relating to reproductive disorders, cancers and neurological diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and neurological disorders, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. reproductive, neurological, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 440 as residues: Asp-47 to Asp-63, His-75 to Tyr-80, Pro-83 to Tyr-89.

The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and treatment of reproductive disorders such as endometrial tumors. Expression of this gene in tissues of the CNS, and its strong homology to Neuritin, suggest that the protein product from this gene is also useful in the treatment and diagnosis of neurological disorders and in the regeneration of neural tissues, e.g., following injury.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:202 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1603 of SEQ ID NO:202, b is an

integer of 15 to 1617, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:202, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 193

The translation product of this gene shares sequence homology with tenascin, which is thought to be important in development. The translation product of this gene is believed to be a ligand of the fibroblast growth factor family. FGF ligand activity is known in the art and can be assayed by methods known in the art and disclosed elsewhere herein.

Northern analysis indicates that a 2.5 kb band is expressed in brain and lung. It has also been discovered that this gene is expressed in endometrial tumor, synovial sarcoma, pancreas tumor, fetal lung, retinal, and immune tissues (e.g., bone marrow)

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancers, growth disorders of the brain and lung. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cancer tissues, brain, lung, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. brain, lung, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 441 as residues: Gly-29 to Glu-34, Arg-71 to Arg-76, Thr-176 to Cys-182, Gly-184 to Glu-199. As a preferred embodiment, antibodies that bind said epitopes are encompassed by the invention and may be useful as a cancer diagnostic and/or an agonist/antagonist of the polypeptides of the invention.



Fragments and variants of the polypeptide encoded by this gene (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention). Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. Antibodies that bind polypeptides of the invention would be useful as a cancer diagnostic.

Preferred polypeptide fragments of the invention comprise, or alternatively consist of, the secreted protein having a continuous series of deleted residues from the amino or the carboxy terminus, or both. Particularly, N-terminal deletions of the polypeptide can be described by the general formula m-379 where m is an integer from 2 to 371, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:441. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: A-2 to W-379; R-3 to W-379; R-4 to W-379; S-5 to W-379; A-6 to W-379; F-7 to W-379; P-8 to W-379; A-9 to W-379; A-10 to W-379; A-11 to W-379; L-12 to W-379; W-13 to W-379; L-14 to W-379; W-15 to W-379; S-16 to W-379; I-17 to W-379; L-18 to W-379; L-19 to W-379; C-20 to W-379; L-21 to W-379; L-22 to W-379; A-23 to W-379; L-24 to W-379; R-25 to W-379; A-26 to W-379; E-27 to W-379; A-28 to W-379; G-29 to W-379; P-30 to W-379; P-31 to W-379; Q-32 to W-379; E-33 to W-379; E-34 to W-379; S-35 to W-379; L-36 to W-379; Y-37 to W-379; L-38 to W-379; W-39 to W-379; I-40 to W-379; D-41 to W-379; A-42 to W-379; H-43 to W-379; Q-44 to W-379; A-45 to W-379; R-46 to W-379; V-47 to W-379; L-48 to W-379; I-49 to W-379; G-50 to W-379; F-51 to W-379; E-52 to W-379; E-53 to W-379; D-54 to W-379; I-55 to W-379; L-56 to W-379; I-57 to W-379; V-58 to W-379; S-59 to W-379; E-60 to W-379; G-61 to W-379; K-62 to W-379; M-63 to W-379; A-64 to W-379; P-65 to W-379; F-66 to W-379; T-67 to W-379; H-68 to W-379; D-69 to W-379; F-70 to W-379; R-71 to W-379; K-72 to W-379; A-73 to W-379; Q-74 to W-379; Q-75 to W-379; R-76 to W-379; M-77 to W-379; P-78 to W-379; A-79 to W-379; I-80 to W-379; P-81 to W-379; V-82 to W-379; N-83 to

W-379; I-84to W-379; H-85 to W-379; S-86 to W-379; M-87 to W-379;N-88 to W-379; F-89 to W-379; T-90 to W-379; W-91 toW-379; Q-92 to W-379; A-93 to W-379; A-94 to W-379;G-95 to W-379; Q-96 to W-379; A-97 to W-379; E-98 toW-379; Y-99 to W-379; F-100 to W-379; Y-101 to W-379;E-102 to W-379; F-103 to W-379;  
5 L-104 to W-379; S-105 toW-379; L-106 to W-379; R-107 to W-379; S-108 to W-379;L-109 to W-379; D-110 to W-379; K-111 to W-379; G-112 toW-379; I-113 to W-379; M-114 to W-379; A-115 to W-379;D-116 to W-379; P-117 to W-379; T-118 to W-379; V-119 toW-379; N-120 to W-379; V-121 to W-379; P-122 to W-379;L-123 to W-379; L-124 to W-379; G-125 to W-379; T-126 toW-379; V-127 to W-379;  
10 P-128 to W-379; H-129 to W-379;K-130 to W-379; A-131 to W-379; S-132 to W-379; V-133 toW-379; V-134 to W-379; Q-135 to W-379; V-136 to W-379;G-137 to W-379; F-138 to W-379; P-139 to W-379; C-140 toW-379; L-141 to W-379; G-142 to W-379; K-143 to W-379;Q-144 to W-379; D-145 to W-379; G-146 to W-379; V-147 toW-379; A-148 to W-379; A-149 to W-379; F-150 to W-379;E-151 to W-379;  
15 V-152 to W-379; D-153 to W-379; V-154 toW-379; I-155 to W-379; V-156 to W-379; M-157 to W-379;N-158 to W-379; S-159 to W-379; E-160 to W-379; G-161 toW-379; N-162 to W-379; T-163 to W-379; I-164 to W-379;L-165 to W-379; Q-166 to W-379; T-167 to W-379; P-168 toW-379; Q-169 to W-379; N-170 to W-379; A-171 to W-379;I-172 to W-379; F-173 to W-379; F-174 to W-379; K-175 toW-379; T-176 to W-379; C-177 to W-379; Q-178 to W-379;Q-179 to W-379; A-180 to W-379; E-181 to W-379; C-182 toW-379; P-183 to W-379; G-184 to W-379; G-185 to W-379;C-186 to W-379; R-187 to W-379; N-188 to W-379; G-189 toW-379; G-190 to W-379; F-191 to W-379; C-192 to W-379;N-193 to W-379; E-194 to W-379; R-195 to W-379; R-196 toW-379; I-197 to W-379; C-198 to W-379; E-199 to W-379;C-200  
25 to W-379; P-201 to W-379; D-202 to W-379; G-203 toW-379; F-204 to W-379; H-205 to W-379; G-206 to W-379;P-207 to W-379; H-208 to W-379; C-209 to W-379; E-210 toW-379; K-211 to W-379; A-212 to W-379; L-213 to W-379;C-214 to W-379; T-215 to W-379; P-216 to W-379; R-217 toW-379; C-218 to W-379; M-219 to W-379; N-220 to W-379;G-221 to W-379; G-222 to W-379; L-223 to W-379; C-224  
30 toW-379; V-225 to W-379; T-226 to W-379; P-227 to W-379;G-228 to W-379; F-229 to W-379; C-230 to W-379; I-231 toW-379; C-232 to W-379; P-233 to W-379; P-234 to W-379;G-235 to W-379; F-236 to W-379; Y-237 to W-379; G-238 toW-379; V-

239 to W-379; N-240 to W-379; C-241 to W-379; D-242 to W-379; K-243 to W-379; A-244 to W-379; N-245 to W-379; C-246 to W-379; S-247 to W-379; T-248 to W-379; T-249 to W-379; C-250 to W-379; F-251 to W-379; N-252 to W-379; G-253 to W-379; G-254 to W-379; T-255 to W-379; C-256 to W-379; F-257 to W-379; Y-258 to W-379; P-259 to W-379; G-260 to W-379; K-261 to W-379; C-262 to W-379; I-263 to W-379; C-264 to W-379; P-265 to W-379; P-266 to W-379; G-267 to W-379; L-268 to W-379; E-269 to W-379; G-270 to W-379; E-271 to W-379; Q-272 to W-379; C-273 to W-379; E-274 to W-379; I-275 to W-379; S-276 to W-379; K-277 to W-379; C-278 to W-379; P-279 to W-379; Q-280 to W-379; P-281 to W-379; C-282 to W-379; R-283 to W-379; N-284 to W-379; G-285 to W-379; G-286 to W-379; K-287 to W-379; C-288 to W-379; I-289 to W-379; G-290 to W-379; K-291 to W-379; S-292 to W-379; K-293 to W-379; C-294 to W-379; K-295 to W-379; C-296 to W-379; S-297 to W-379; K-298 to W-379; G-299 to W-379; Y-300 to W-379; Q-301 to W-379; G-302 to W-379; D-303 to W-379; L-304 to W-379; C-305 to W-379; S-306 to W-379; K-307 to W-379; P-308 to W-379; V-309 to W-379; C-310 to W-379; E-311 to W-379; P-312 to W-379; G-313 to W-379; C-314 to W-379; G-315 to W-379; A-316 to W-379; H-317 to W-379; G-318 to W-379; T-319 to W-379; C-320 to W-379; H-321 to W-379; E-322 to W-379; P-323 to W-379; N-324 to W-379; K-325 to W-379; C-326 to W-379; Q-327 to W-379; C-328 to W-379; Q-329 to W-379; E-330 to W-379; G-331 to W-379; W-332 to W-379; H-333 to W-379; G-334 to W-379; R-335 to W-379; H-336 to W-379; C-337 to W-379; N-338 to W-379; K-339 to W-379; R-340 to W-379; Y-341 to W-379; E-342 to W-379; A-343 to W-379; S-344 to W-379; L-345 to W-379; I-346 to W-379; H-347 to W-379; A-348 to W-379; L-349 to W-379; R-350 to W-379; P-351 to W-379; A-352 to W-379; G-353 to W-379; A-354 to W-379; Q-355 to W-379; L-356 to W-379; R-357 to W-379; Q-358 to W-379; H-359 to W-379; T-360 to W-379; P-361 to W-379; S-362 to W-379; L-363 to W-379; K-364 to W-379; K-365 to W-379; A-366 to W-379; E-367 to W-379; E-368 to W-379; R-369 to W-379; R-370 to W-379; D-371 to W-379; P-372 to W-379; P-373 to W-379; and E-374 to W-379 of SEQ ID NO: 441. Polypeptides encoded by these polynucleotides are also encompassed by the invention. Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to

these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind ligand, ability to generate antibodies, ability to bind antibodies) may still be retained. For example the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the polypeptide shown in SEQ ID NO:441, as described by the general formula 1-n, where n is an integer from 6 to 378, where n corresponds to the position of the amino acid residue identified in SEQ ID NO:441. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, an amino acid sequence selected from the group: M-1 to I-378; M-1 to Y-377; M-1 to N-376; M-1 to S-375; M-1 to E-374; M-1 to P-373; M-1 to P-372; M-1 to D-371; M-1 to R-370; M-1 to R-369; M-1 to E-368; M-1 to E-367; M-1 to A-366; M-1 to K-365; M-1 to K-364; M-1 to L-363; M-1 to S-362; M-1 to P-361; M-1 to T-360; M-1 to H-359; M-1 to Q-358; M-1 to R-357; M-1 to L-356; M-1 to Q-355; M-1 to A-354; M-1 to G-353; M-1 to A-352; M-1 to P-351; M-1 to R-350; M-1 to L-349; M-1 to A-348; M-

1 to H-347; M-1 toI-346; M-1 to L-345; M-1 to S-344; M-1 to A-343; M-1 toE-342;  
M-1 to Y-341; M-1 to R-340; M-1 to K-339; M-1 toN-338; M-1 to C-337; M-1 to H-  
336; M-1 to R-335; M-1 toG-334; M-1 to H-333; M-1 to W-332; M-1 to G-331; M-1  
toE-330; M-1 to Q-329; M-1 to C-328; M-1 to Q-327; M-1 toC-326; M-1 to K-325;  
5 M-1 to N-324; M-1 to P-323; M-1 toE-322; M-1 to H-321; M-1 to C-320; M-1 to T-  
319; M-1 toG-318; M-1 to H-317; M-1 to A-316; M-1 to G-315; M-1 toC-314; M-1  
to G-313; M-1 to P-312; M-1 to E-311; M-1 toC-310; M-1 to V-309; M-1 to P-308;  
M-1 to K-307; M-1 toS-306; M-1 to C-305; M-1 to L-304; M-1 to D-303; M-1 toG-  
302; M-1 to Q-301; M-1 to Y-300; M-1 to G-299; M-1 toK-298; M-1 to S-297; M-1  
10 to C-296; M-1 to K-295; M-1 toC-294; M-1 to K-293; M-1 to S-292; M-1 to K-291;  
M-1 toG-290; M-1 to I-289; M-1 to C-288; M-1 to K-287; M-1 toG-286; M-1 to G-  
285; M-1 to N-284; M-1 to R-283; M-1 toC-282; M-1 to P-281; M-1 to Q-280; M-1  
to P-279; M-1 toC-278; M-1 to K-277; M-1 to S-276; M-1 to I-275; M-1 toE-274; M-  
1 to C-273; M-1 to Q-272; M-1 to E-271; M-1 toG-270; M-1 to E-269; M-1 to L-268;  
15 M-1 to G-267; M-1 toP-266; M-1 to P-265; M-1 to C-264; M-1 to I-263; M-1 toC-  
262; M-1 to K-261; M-1 to G-260; M-1 to P-259; M-1 toY-258; M-1 to F-257; M-1  
to C-256; M-1 to T-255; M-1 toG-254; M-1 to G-253; M-1 to N-252; M-1 to F-251;  
M-1 toC-250; M-1 to T-249; M-1 to T-248; M-1 to S-247; M-1 toC-246; M-1 to N-  
245; M-1 to A-244; M-1 to K-243; M-1 toD-242; M-1 to C-241; M-1 to N-240; M-1  
20 to V-239; M-1 toG-238; M-1 to Y-237; M-1 to F-236; M-1 to G-235; M-1 toP-234;  
M-1 to P-233; M-1 to C-232; M-1 to I-231; M-1 toC-230; M-1 to F-229; M-1 to G-  
228; M-1 to P-227; M-1 toT-226; M-1 to V-225; M-1 to C-224; M-1 to L-223; M-1  
toG-222; M-1 to G-221; M-1 to N-220; M-1 to M-219; M-1 toC-218; M-1 to R-217;  
M-1 to P-216; M-1 to T-215; M-1 toC-214; M-1 to L-213; M-1 to A-212; M-1 to K-  
25 211; M-1 toE-210; M-1 to C-209; M-1 to H-208; M-1 to P-207; M-1 toG-206; M-1 to  
H-205; M-1 to F-204; M-1 to G-203; M-1 toD-202; M-1 to P-201; M-1 to C-200; M-  
1 to E-199; M-1 toC-198; M-1 to I-197; M-1 to R-196; M-1 to R-195; M-1 toE-194;  
M-1 to N-193; M-1 to C-192; M-1 to F-191; M-1 toG-190; M-1 to G-189; M-1 to N-  
188; M-1 to R-187; M-1 toC-186; M-1 to G-185; M-1 to G-184; M-1 to P-183; M-1  
30 toC-182; M-1 to E-181; M-1 to A-180; M-1 to Q-179; M-1 toQ-178; M-1 to C-177;  
M-1 to T-176; M-1 to K-175; M-1 toF-174; M-1 to F-173; M-1 to I-172; M-1 to A-  
171; M-1 toN-170; M-1 to Q-169; M-1 to P-168; M-1 to T-167; M-1 toQ-166; M-1 to

L-165; M-1 to I-164; M-1 to T-163; M-1 to N-162; M-1 to G-161; M-1 to E-160; M-1  
 to S-159; M-1 to N-158; M-1 to M-157; M-1 to V-156; M-1 to I-155; M-1 to V-154;  
 M-1 to D-153; M-1 to V-152; M-1 to E-151; M-1 to F-150; M-1 to A-149; M-1 to A-  
 148; M-1 to V-147; M-1 to G-146; M-1 to D-145; M-1 to Q-144; M-1 to K-143; M-1  
 5 to G-142; M-1 to L-141; M-1 to C-140; M-1 to P-139; M-1 to F-138; M-1 to G-137;  
 M-1 to V-136; M-1 to Q-135; M-1 to V-134; M-1 to V-133; M-1 to S-132; M-1 to A-  
 131; M-1 to K-130; M-1 to H-129; M-1 to P-128; M-1 to V-127; M-1 to T-126; M-1 to  
 G-125; M-1 to L-124; M-1 to L-123; M-1 to P-122; M-1 to V-121; M-1 to N-120; M-  
 1 to V-119; M-1 to T-118; M-1 to P-117; M-1 to D-116; M-1 to A-115; M-1 to M-114;  
 10 M-1 to I-113; M-1 to G-112; M-1 to K-111; M-1 to D-110; M-1 to L-109; M-1 to S-  
 108; M-1 to R-107; M-1 to L-106; M-1 to S-105; M-1 to L-104; M-1 to F-103; M-1  
 to E-102; M-1 to Y-101; M-1 to F-100; M-1 to Y-99; M-1 to E-98; M-1 to A-97; M-1  
 to Q-96; M-1 to G-95; M-1 to A-94; M-1 to A-93; M-1 to Q-92; M-1 to W-91; M-1 to  
 T-90; M-1 to F-89; M-1 to N-88; M-1 to M-87; M-1 to S-86; M-1 to H-85; M-1 to I-  
 15 84; M-1 to N-83; M-1 to V-82; M-1 to P-81; M-1 to I-80; M-1 to A-79; M-1 to P-78;  
 M-1 to M-77; M-1 to R-76; M-1 to Q-75; M-1 to Q-74; M-1 to A-73; M-1 to K-72; M-  
 1 to R-71; M-1 to F-70; M-1 to D-69; M-1 to H-68; M-1 to T-67; M-1 to F-66; M-1 to  
 P-65; M-1 to A-64; M-1 to M-63; M-1 to K-62; M-1 to G-61; M-1 to E-60; M-1 to S-  
 59; M-1 to V-58; M-1 to I-57; M-1 to L-56; M-1 to I-55; M-1 to D-54; M-1 to E-53;  
 20 M-1 to E-52; M-1 to F-51; M-1 to G-50; M-1 to I-49; M-1 to L-48; M-1 to V-47; M-1  
 to R-46; M-1 to A-45; M-1 to Q-44; M-1 to H-43; M-1 to A-42; M-1 to D-41; M-1 to  
 I-40; M-1 to W-39; M-1 to L-38; M-1 to Y-37; M-1 to L-36; M-1 to S-35; M-1 to E-  
 34; M-1 to E-33; M-1 to Q-32; M-1 to P-31; M-1 to P-30; M-1 to G-29; M-1 to A-28;  
 M-1 to E-27; M-1 to A-26; M-1 to R-25; M-1 to L-24; M-1 to A-23; M-1 to L-22; M-1  
 25 to L-21; M-1 to C-20; M-1 to L-19; M-1 to L-18; M-1 to I-17; M-1 to S-16; M-1 to  
 W-15; M-1 to L-14; M-1 to W-13; M-1 to L-12; M-1 to A-11; M-1 to A-10; M-1 to A-  
 9; M-1 to P-8; M-1 to F-7; and M-1 to A-6 of SEQ ID NO:441. Polypeptides  
 encoded by these polynucleotides are also encompassed by the invention. Moreover,  
 fragments and variants of these polypeptides (such as, for example, fragments as  
 30 described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or  
 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
 which hybridizes, under stringent conditions, to the polynucleotide encoding these

polypeptides , or the complement there of are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

5           The tissue distribution in brain and lung, combined with the homology to tenascin indicates that the protein product of this gene is useful for diagnosis and treatment of cancers. Alternatively, given the tissue distribution indicated by Northern analysis, the translation product of this gene is thought to be a growth factor functioning in the brain and lung that may be useful in treating neurodegeneration and  
10   lung disorder. For example, the protein product of this gene is useful for the detection and treatment of disorders associated with developing lungs, particularly in premature infants where the lungs are the last tissues to develop. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and intervention of lung tumors, since the gene may be involved in the regulation of  
15   cell division. Additionally, expression in the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's.

20           Polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of hematopoietic related disorders such as anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in  
25   Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia. The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene  
30   product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:203 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1960 of SEQ ID NO:203, b is an integer of 15 to 1974, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:203, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 194**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: MNSAAGFSHLDRRERVVLKLGESFEKQPRCASTLC (SEQ ID NO:1203).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in fetal human lung and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lung development and respiratory disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for



differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the respiratory system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. respiratory, immune, cancerous and wounded tissues) or  
 5 bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal lung and neutrophils indicates that the protein  
 10 product of this gene is useful for the diagnosis and treatment of lung and immunity related diseases, for example, lung cancer, viral, fungal or bacterial infections (e.g. lesions caused by tuberculosis), inflammation (e.g. pneumonia), metabolic lesions etc. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the detection and treatment of disorders associated with developing  
 15 lungs, particularly in premature infants where the lungs are the last tissues to develop. The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and intervention of lung tumors, since the gene may be involved in the regulation of cell division, particularly since it is expressed in fetal tissue. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker  
 20 and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:204 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
 25 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1043 of SEQ ID NO:204, b is an integer of 15 to 1057, where both a and b correspond to the positions of nucleotide  
 30 residues shown in SEQ ID NO:204, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 195

This gene is expressed primarily in breast lymph node, and to a lesser extent in synovial tissues.

5 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune and skeletal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
10 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system and skeletal system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another  
15 tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in breast lymph node and synovium indicates that the protein product of this gene is useful for the diagnosis and treatment of immune and  
20 skeletal disorders. Furthermore, this gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or  
25 immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood  
30 lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. The expression of this

gene product in synovium indicates a role in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis as well as disorders afflicting connective tissues (e.g. arthritis, trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:205 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 707 of SEQ ID NO:205, b is an integer of 15 to 721, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:205, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 196**

The gene encoding the disclosed cDNA is thought to reside on chromosome 5. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 5. The translation product of this gene shares sequence homology with human M-phase phosphoprotein 4, which is thought to be important in the phosphorylation and signal transduction processes.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

TIYPTEEELQAVQKIVSITERALKLVSD (SEQ ID NO:1204),  
 RALKGVLRVGVLAKEGGLLRGDRNVNLVLLC (SEQ ID NO:1205),  
 ALAALRHAKWFAQRANGLQSCVIIIIRLDLCQRVPTWS (SEQ ID NO:1206),  
 GDALRRVFECISSGIL (SEQ ID NO:1207), LAFRQIHKVLGMDPLP (SEQ ID  
 5 NO:1208), and/or

TIYPTEEELQAVQKIVSITERALKLVSDSLSEHEKNKNKEGDDKKEGGKDRAL  
 KGVLRVGVLAKEGGLLRGDRNVNLVLLCSEKPSKTLLSRIAENLPKQLAVISPE  
 KYDIKCAVSEAAIILNSCVEPKMQVTITLTSPHREENMREGDVTSGMVKDPPD  
 VLDRQKCLDALAALRHAKWFAQRANGLQSCVIIIIRLDLCQRVPTWSDFPS  
 10 WAMELLVEKAISSASSPQSPGDALRRVFECISSGILKGSPGLLDPCFKDPFDTL  
 ATMTDQQREDITSSAQFALRLLAFRQIHKVLGMDPLPQMSQRFNIHNNRKRR  
 RDSGVDGFEAEGKKDKKDYDNF (SEQ ID NO:1209), MERHPKKKMCS  
 (SEQ ID NO:1210), and/or GENSSSDFPLFLFYFLVALASPIFVSFIN (SEQ ID  
 NO:1211). Moreover, fragments and variants of these polypeptides (such as, for  
 15 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides) are encompassed by the invention. Antibodies that bind  
 polypeptides of the invention are also encompassed by the invention. Polynucleotides  
 20 encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human hippocampus, and to a lesser extent  
 in prostate and human frontal cortex.

Polynucleotides and polypeptides of the invention are useful as reagents for  
 differential identification of the tissue(s) or cell type(s) present in a biological sample  
 25 and for diagnosis of diseases and conditions which include, but are not limited to,  
 disorders related to the reproductive and nervous systems. Similarly, polypeptides  
 and antibodies directed to these polypeptides are useful in providing immunological  
 probes for differential identification of the tissue(s) or cell type(s). For a number of  
 disorders of the above tissues or cells, particularly of the reproductive and nervous  
 30 systems, expression of this gene at significantly higher or lower levels may be  
 routinely detected in certain tissues or cell types (e.g. reproductive, CNS, cancerous  
 and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial

fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID

5 NO: 444 as residues: Arg-13 to Asp-21, Lys-28 to Lys-38, Val-76 to Asp-81, Ser-99 to Ala-107, Pro-130 to Phe-136, Thr-143 to Ile-150, Pro-176 to Phe-182, Asn-186 to Gly-196, Ala-202 to Phe-214.

10 The tissue distribution in human hippocampus, prostate, and frontal cortex, combined with the homology to human M-phase phosphoprotein 4 indicates that the protein product of this gene is useful for the diagnosis and treatment of reproductive and nervous system disorders. Furthermore, elevated expression of this gene product within the frontal cortex of the brain indicates that it may be involved in neuronal survival; synapse formation; conductance; neural differentiation, etc. Such involvement may impact many processes, such as learning and cognition. It may also  
15 be useful in the treatment of such neurodegenerative disorders as schizophrenia; ALS; or Alzheimer's. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

20 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:206 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
25 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2451 of SEQ ID NO:206, b is an integer of 15 to 2465, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:206, and where b is greater than or equal to a + 14.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 197**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

MGSQHSAAARPSSCRRKQEDDRDG (SEQ ID NO:1212),

LLAEREQEEAIAQFPYVEFTGRDSITCLTC (SEQ ID NO:1213), and/or

5 QGTGYIPTEQVNELVALI PHSDQRLRPQRTKQYV (SEQ ID NO:1214).

Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
10 encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in human primary breast cancer, and to a lesser extent, in human adult spleen, Hodgkin's lymphoma I, and salivary gland.

15 Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer, as well as immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
20 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly cancers and the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell  
25 sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 445 as residues: Ser-126 to Gly-138.

30 The tissue distribution in tumors of breast origins indicates that the protein product of this gene is useful for the diagnosis and intervention of these tumors, in addition to other tumors where expression has been indicated. Furthermore, the

expression in hematopoietic cells and tissues indicates that this protein may play a role in the proliferation, differentiation, and/or survival of hematopoietic cell lineages. In such an event, this gene may be useful in the treatment of lymphoproliferative disorders, and in the maintenance and differentiation of various hematopoietic lineages from early hematopoietic stem and committed progenitor cells. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:207 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1466 of SEQ ID NO:207, b is an integer of 15 to 1480, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:207, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 198**

This gene is expressed primarily in monocytes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, blood cell disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample

taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in monocytes indicates that the protein product of this gene is useful for the diagnosis and treatment of blood cell disorders. Furthermore, expression of this gene product in monocytes also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:208 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 858 of SEQ ID NO:208, b is an integer of 15 to 872, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:208, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 199**

The gene encoding the disclosed cDNA is thought to reside on chromosome 6. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 6.

This gene is expressed primarily in human ovary and synovia, and to a lesser extent in human 8 week whole embryo.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, reproductive and developmental disorders. Similarly, polypeptides and antibodies



directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and developmental systems, expression of this gene at significantly higher or lower levels may be routinely

5 detected in certain tissues or cell types (e.g. reproductive, developmental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 The tissue distribution in human ovary and human 8 week whole embryo indicates that the protein product of this gene is useful for the diagnosis and treatment of reproductive and developmental disorders. Similarly, expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in

15 the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or

20 immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:209 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

25 excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1765 of SEQ ID NO:209, b is an integer of 15 to 1779, where both a and b correspond to the positions of nucleotide

30 residues shown in SEQ ID NO:209, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 200

The gene encoding the disclosed cDNA is thought to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8. The translation product of this gene shares limited sequence homology with collagen proline rich domain.

This gene is expressed primarily in CNS.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurological diseases. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. CNS, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 448 as residues: Pro-35 to Asp-41.

The tissue distribution in tissues of the central nervous system indicates that the protein product of this gene is useful for the diagnosis and treatment of neurological diseases and disorders, such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette's Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders. Protein, as well as, antibodies

directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:210 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2096 of SEQ ID NO:210, b is an integer of 15 to 2110, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:210, and where b is greater than or equal to a + 14.

## 15 FEATURES OF PROTEIN ENCODED BY GENE NO: 201

The translation product of this gene shares homology with a mammalian histone H1a protein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

ARLNVGRESLKREMLKSQGVKVSSESPMGARHSSWPEGAAFCCKKVQGAQMQFPPRR (SEQ ID NO:1215), ARLNVGRESLKREML (SEQ ID NO:1216), LKSQGVKVSSESPMGARHSSW (SEQ ID NO:1217), AFCCKKVQGAQMQFPPRR (SEQ ID NO:1218), and/or AFCCKKVQGAQMQFPPRR (SEQ ID NO:1219). Moreover,

fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention. (See Genbank Accession No. pir|S24178).

This gene is expressed primarily in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to,

5 immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell  
10 types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

15 The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the diagnosis and treatment of immune disorders. Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in vital immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and  
20 leukemia. Furthermore, expression of this gene product in neutrophils also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
25 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:211 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
30 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 924 of SEQ ID NO:211, b is an

integer of 15 to 938, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:211, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 202

This gene is expressed primarily in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the diagnosis and treatment of immune disorders. Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia. Furthermore, expression of this gene product in neutrophils also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:212 and may have been publicly available prior to conception

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of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

5 formula of a-b, where a is any integer between 1 to 1537 of SEQ ID NO:212, b is an integer of 15 to 1551, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:212, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 203

This gene is expressed primarily in neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample

15 and for diagnosis of diseases and conditions which include, but are not limited to, infectious disorders, immune disorders, and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression

20 of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from

25 an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 451 as residues: Thr-31 to Lys-36.

The tissue distribution in neutrophils indicates that the protein product of this gene is useful for the diagnosis and treatment of infectious disorders, immune

30 disorders, and cancers. Since the gene is expressed in cells of lymphoid origin, the natural gene product may be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune

deficiency diseases such as AIDS, and leukemia. Expression of this gene product in neutrophils also strongly indicates a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:213 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 983 of SEQ ID NO:213, b is an integer of 15 to 997, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:213, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 204**

The gene encoding the disclosed cDNA is thought to reside on chromosome 16. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 16. The translation product of this gene shares sequence homology with lactate dehydrogenase, which is thought to be important in lactate metabolism.

This gene is expressed primarily in human tonsils, and to a lesser extent, in spleen, and neutrophils.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders, infectious disorders, and cancers. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of

disorders of the above tissues or cells, particularly of the immune disorders, infectious disorders, and cancers, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. tonsils, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 452 as residues: Gly-7 to Ser-12.

The tissue distribution in human tonsils, spleen, and neutrophils, combined with the homology to lactate dehydrogenase gene indicates that the protein product of this gene is useful for the diagnosis and treatment of immune disorders, infectious disorders, and cancers. Furthermore, expression of this gene product in tonsils indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:214 and may have been publicly available prior to conception



of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general

5 formula of a-b, where a is any integer between 1 to 1482 of SEQ ID NO:214, b is an integer of 15 to 1496, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:214, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 205**

The translation product of this gene shares sequence homology with Gcap1 protein which is developmentally regulated in brain.

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In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

NFFFVCLFKSSLRLVNSSYTPILCVL (SEQ ID NO:1220). Moreover, fragments and variants of this polypeptide (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to

20

these polypeptides and polypeptides encoded by the polynucleotide which hybridize, under stringent conditions, to the polynucleotide encoding this polypeptide are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding this polypeptide are also encompassed by the invention.

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The gene encoding the disclosed cDNA is thought to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in placenta and endometrial tumors.

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Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, vasculogenesis/angiogenesis and tumorigenesis. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the vascular system and tumors, expression of this gene at significantly higher or lower levels may be routinely  
 5 detected in certain tissues or cell types (e.g. placental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

10 Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 453 as residues: Lys-9 to Gln-16.

The tissue distribution placenta and endometrial tumors, combined with the homology to Gcap1 protein indicates that the protein product of this gene is useful for the diagnosis and treatment of disorders or dysfunctions of the vascular system, which  
 15 include, but are not limited to atherosclerosis, hypertension, embolism, thrombosis, microvascular disease, aneurysm, or stroke, or tumorigenesis. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment and  
 20 maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells or within the  
 25 circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:215 and may have been publicly available prior to conception

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of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1294 of SEQ ID NO:215, b is an integer of 15 to 1308, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:215, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 206

The translation product of this gene shares sequence homology with a *C. elegans* protein of unknown function (F23B2.4 [*Caenorhabditis elegans*]).

In specific embodiments, polypeptides of the invention comprise, or alternatively

15 consist of, the following amino acid sequences:

VQVLEQLTNNVAESRFNDAAYYYWMLSMQCLDIAQD (SEQ ID NO:1221),

PAQKDTMLGKFYHFQRLAELYHGYHAHRHTEDP (SEQ ID NO:1222),

LAKQSKALGAYRLARHAYDKLRGLYIP (SEQ ID NO:1223),

ARFQKSIELGTLTIRAKPFHDSEELVPLCYRCSTNN (SEQ ID NO:1224),

20 PLLNNLGNVCINCRQPFIFSASSYDVLHLVEFYLEEGITDEEAISLIDLEVLRPK

RDDRQLEICKQQLPDSCG (SEQ ID NO:1225)

MPYAQWLAENDRFEEAQKAFHKAGRQREA (SEQ ID NO:1226), and/or

FSVHRPETLFNISRFLHSLPKDTPSGISKVKILFT (SEQ ID NO:1227).

Moreover, fragments and variants of these polypeptides (such as, for example,

25 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the

polynucleotide which hybridizes, under stringent conditions, to the polynucleotide

encoding these polypeptides) are encompassed by the invention. Antibodies that bind

polypeptides of the invention are also encompassed by the invention. Polynucleotides

30 encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in testes.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, male reproductive and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. testes, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, seminal fluid, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in testes indicates that the protein product of this gene is useful for the treatment of male reproductive and endocrine disorders. Furthermore, the tissue distribution indicates that the protein product of this gene is useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists/agonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:216 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is

cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1691 of SEQ ID NO:216, b is an integer of 15 to 1705, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:216, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 207

This gene is expressed in fetal lung.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, lung diseases such as cystic fibrosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the respiratory system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g. respiratory, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Predicted epitopes include those comprising a sequence shown in SEQ ID NO: 455 as residues: Tyr-49 to Cys-54.

The tissue distribution in fetal lung indicates that the protein product of this gene is useful for the detection and treatment of disorders associated with developing lungs, particularly in premature infants where the lungs are the last tissues to develop. The tissue distribution indicates that the protein product of this gene is useful for the diagnosis and intervention of lung tumors, since the gene may be involved in the regulation of cell division, particularly since it is expressed in fetal tissue. Protein, as

well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
5 related to SEQ ID NO:217 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 985 of SEQ ID NO:217, b is an integer of 15 to 999, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:217, and where b is greater than or equal to a + 14.

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Table 1

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of 5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HLHDS67	97979 03/27/97	Uni-ZAP XR	11	2526	427	2526	458	458	249	1	30	31	30
2	HLHDZ58	97979 03/27/97	Uni-ZAP XR	12	1131	1	1131	129	129	250	1	14	15	115
3	HLMMJ13	97979 03/27/97	Lambda ZAP II	13	941	39	941	62	62	251	1	44	45	102
3	HLMMJ13	97979 03/27/97	Lambda ZAP II	218	941	39	941	245	245	456	1	35	36	41
4	HLTEI25	97979 03/27/97	Uni-ZAP XR	14	843	1	843	155	155	252	1	19	20	42
5	HMSJX24	97979 03/27/97	Uni-ZAP XR	15	1018	1	1018	90	90	253	1	18	19	36
6	HNFE65	97979 03/27/97	Uni-ZAP XR	16	661	1	661	76	76	254	1	28	29	127
7	HNHDX07	97979 03/27/97	Uni-ZAP XR	17	553	1	553	106	106	255	1	23	24	66
8	HNHGC82	97979 03/27/97	Uni-ZAP XR	18	869	1	869	101	101	256	1	21	22	68

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
9	HNHGO09	97979 03/27/97	Uni-ZAP XR	19	959	1	959	176	176	257	1	21	22	43
10	HOUBE18	97979 03/27/97	Uni-ZAP XR	20	1446	1	1446	101	101	258	1	27	28	50
11	HOUDL69	97979 03/27/97	Uni-ZAP XR	21	1471	579	1460	692	692	259	1	31	32	42
12	HPMFI71	97979 03/27/97	Uni-ZAP XR	22	1402	242	1402	401	401	260	1	32	33	60
13	HPMGQ55	97979 03/27/97	Uni-ZAP XR	23	1047	1	1047	164	164	261	1	26	27	35
14	HPQAC69	97979 03/27/97	Lambda ZAP II	24	990	1	988	82	82	262	1	20	21	37
15	HPTBB03	97979 03/27/97	Uni-ZAP XR	25	1208	350	1173	398	398	263	1	29	30	210
16	HPTWA66	97979 03/27/97	pBluescript	26	1922	1381	1922	24	24	264	1	33	34	547
16	HPTWA66	97979 03/27/97	pBluescript	219	575	1	575	148	148	457	1	22	23	65
17	HPTWC08	97979 03/27/97	pBluescript	27	1951	1422	1874	219	219	265	1	19	20	299
18	HRGCZ46	97979 03/27/97	Uni-ZAP XR	28	3989	2635	3989		2748	266	1	16	17	39



Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
19	HSAVU34	97979 03/27/97	Uni-ZAP XR	29	3735	2966	3735	272	272	267	1	30	31	594
19	HSAVU34	97979 03/27/97	Uni-ZAP XR	220	3018	1929	3018	26	26	458	1	1	2	156
20	HSDFW61	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	30	1667	59	1625	138	138	268	1	32	33	130
21	HSDGP60	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	31	1408	1	1408	285	285	269	1			20
22	HSOAJ55	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	32	3186	2402	3186	302	302	270	1	43	44	159
22	HSOAJ55	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	221	2031	1273	2031	1285	1285	459	1	29	30	30

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
23	HSQEO84	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	33	971	13	971	91	91	271	1	19	20	218
23	HSQEO84	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	222	968	8	968	86	86	460	1	20	21	56
24	HSXAM05	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	34	1792	369	1792	470	470	272	1	26	27	49
25	HSXAS67	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	35	896	1	896	96	96	273	1	32	33	121
26	HTDAF28	97974 04/04/97 209080 05/29/97	pSport1	36	912	1	912	38	38	274	1	22	23	87

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
27	HTEGQ64	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	37	1382	67	1382	271	271	275	1			25
28	HTGEU09	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	38	872	1	872	74	74	276	1	18	19	28
29	HTOAM21	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	39	812	1	812	41	41	277	1	30	31	43
30	HTPBW79	209511 12/03/97	Uni-ZAP XR	40	1515	118	1507	302	302	278	1	24	25	362
30	HTSEV09	97974 04/04/97 209080 05/29/97	pBluescript	223	1404	1	1265	92	92	461	1	19	20	415
31	HJPCD40	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	41	704	22	704		117	279	1	18	19	127

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
32	HTWBY48	97974 04/04/97 209080 05/29/97	pSport1	42	1094	1	1094	32	32	280	1	34	35	53
33	HTWCI46	97974 04/04/97 209080 05/29/97	pSport1	43	1821	892	1647	56	56	281	1	26	27	29
34	HTXGI75	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	44	1024	30	1024	167	167	282	1	20	21	25
35	HWTBF59	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	45	983	779	983	85	85	283	1	30	31	221
35	HWTBF59	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	224	707	488	707	514	514	462	1	41	42	64

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
36	HADAE74	97974 04/04/97 209080 05/29/97	pSport1	46	2421	664	1587	2110	2110	284	1	33	34	40
37	HAGFB60	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	47	840	1	840	97	97	285	1	30	31	48
38	HATEF60	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	48	2432	1193	2246	1491	1491	286	1	17	18	51
39	HBMSN25	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	49	1742	1165	1742	1207	1207	287	1	23	24	31
40	HCDAR68	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	50	1487	181	1455	325	325	288	1	35	36	56

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
41	HCE3J79	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	51	1328	251	1328	525	525	289	1			21
42	HMDAN54	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	52	1856	725	1853	928	928	290	1	33	34	50
43	HCECA49	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	53	1558	310	1408	109	109	291	1	30	31	98
44	HCEEC15	97974 04/04/97 209080 05/29/97	Uni-ZAP XR	54	948	1	948	9	9	292	1	23	24	65
45	HCESF40	97974 04/04/97 209080 05/29/97	pBluescript	55	990	99	990	193	193	293	1	32	33	256

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
45	HCESF40	97974 04/04/97 209080 05/29/97	pBluescript	225	1384	99	1384	193	193	463	1	32	33	205
46	HCFMV39	97974 04/04/97 209080 05/29/97	pSport1	56	1603	1	1296	96	96	294	1	29	30	102
47	HCM SX86	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	57	1052	5	786	12	12	295	1	28	29	32
48	HCNAP62	97975 04/04/97 209081 05/29/97	Lambda ZAP II	58	814	1	558	93	93	296	1	22	23	42
49	HCR AF32	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	59	1215	257	1215		356	297	1	19	20	20

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
50	HCUDC07	97975 04/04/97 209081 05/29/97	ZAP Express	60	478	1	478	147	147	298	1	36	37	69
51	HCWBB42	97975 04/04/97 209081 05/29/97	ZAP Express	61	618	1	618	212	212	299	1	35	36	74
52	HDTAB05	97975 04/04/97 209081 05/29/97	pCMVSPORT 2.0	62	751	1	751	257	257	300	1	21	22	32
53	HE2AV74	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	63	780	283	780		433	301	1			16
54	HE2AY71	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	64	588	21	588	169	169	302	1			16



Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
55	HE2GS36	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	65	945	1	349	520	520	303	1	39	40	111
55	HE2GS36	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	226	774	272	774	445	445	464	1			37
56	HE2OF09	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	66	1866	1313	1866	1596	1596	304	1			11
57	HE6EU50	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	67	1152	117	686	237	237	305	1	20	21	34
58	HE9HU17	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	68	2483	1577	2448	1620	1620	306	1			14

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
59	HE9ND48	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	69	536	1	536	83	83	307	1	36	37	43
60	HEBBW11	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	70	574	97	564	109	109	308	1	55	56	137
60	HEBBW11	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	227	865	647	865		388	465	1	30	31	135
61	HELDY74	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	71	932	1	932	201	201	309	1	17	18	33
62	HEMAE80	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	72	996	1	945	12	12	310	1	24	25	136

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
63	HFEB88	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	73	785	464	785	356	356	311	1	29	30	57
64	HFGAB89	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	74	1069	196	1047	295	295	312	1	32	33	34
65	HFVHY45	97975 04/04/97 209081 05/29/97	pBluescript	75	831	1	831	50	50	313	1	36	37	89
66	HGBAJ93	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	76	590	1	590	233	233	314	1	38	39	94
67	HGBBQ69	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	77	1274	1	1273	105	105	315	1	24	25	43

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
68	HHFCF08	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	78	1133	4	1042	175	175	316	1	23	24	30
69	HHFHJ59	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	79	661	1	661	192	192	317	1	29	30	112
70	HHFHR32	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	80	1378	1	1378	58	58	318	1	25	26	235
71	HHGCN69	97975 04/04/97 209081 05/29/97	Lambda ZAP II	81	1440	298	1440	532	532	319	1	23	24	34
72	HHGDO13	97975 04/04/97 209081 05/29/97	Lambda ZAP II	82	1381	766	1371	993	993	320	1	23	24	34

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
73	HHPFD63	97975 04/04/97 209081 05/29/97	Uni-ZAP XR	83	1706	182	1644	257	257	321	1	24	25	81
74	HHSEG23	97976 04/04/97	Uni-ZAP XR	84	573	1	573	160	160	322	1	18	19	71
75	HJPAV06	97976 04/04/97	Uni-ZAP XR	85	684	199	684	323	323	323	1	27	28	33
76	HKIXL73	97976 04/04/97	pBluescript	86	1036	591	1036	690	690	324	1	32	33	114
77	HKMNC43	97976 04/04/97	pBluescript	87	908	1	908	139	139	325	1	18	19	108
78	HMEJE31	97976 04/04/97	Lambda ZAP II	88	655	1	655	165	165	326	1	33	34	64
79	HMSKS35	97976 04/04/97	Uni-ZAP XR	89	1102	1	1102	228	228	327	1	23	24	49
79	HMSKS35	97976 04/04/97	Uni-ZAP XR	228	1102	1	1102	228	228	466	1	26	27	49
80	HNFAE54	97976 04/04/97	Uni-ZAP XR	90	1533	665	1518	347	347	328	1	26	27	293
81	HNFJH45	97976 04/04/97	Uni-ZAP XR	91	575	1	575	275	275	329	1	30	31	67

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
82	HNGBT31	97976 04/04/97	Uni-ZAP XR	92	639	1	639	224	224	330	1	28	29	104
83	HNGIN60	97976 04/04/97	Uni-ZAP XR	93	858	1	858	239	239	331	1	23	24	58
83	HNGIN60	97976 04/04/97	Uni-ZAP XR	229	744	1	744	225	225	467	1	43	44	70
84	HNGJG84	97976 04/04/97	Uni-ZAP XR	94	526	1	526	268	268	332	1	29	30	38
85	HNHDW42	97976 04/04/97	Uni-ZAP XR	95	426	1	426	168	168	333	1	28	29	71
86	HNHFL57	97976 04/04/97	Uni-ZAP XR	96	844	1	844	98	98	334	1	25	26	61
87	HOGAR52	97977 04/04/97 209082 05/29/97	pCMVSPORT 2.0	97	1985	453	1985	533	533	335	1	17	18	285
88	HOSBZ55	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	98	1416	69	1416	246	246	336	1	32	33	54

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
89	HOSDI92	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	99	1760	1469	1760	934	934	337	1	22	23	59
89	HOSDI92	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	230	1935	141	772		274	468	1	20	21	58
90	HPBCU51	97977 04/04/97 209082 05/29/97	pBluescript SK-	100	599	1	599	86	86	338	1	27	28	119
91	HPCAL49	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	101	784	1	784	113	113	339	1	36	37	38
92	HPFCR13	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	102	404	1	404	266	266	340	1	30	31	46

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
92	HPFCR13	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	231	1035	602	1035	859	859	469	1	32	33	58
93	HPHAC83	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	103	2218	840	2182	1035	1035	341	1	17	18	17
93	HOFNZ45	209568 01/06/98	pCMVSPORT 2.0	232	760	1	728	86	86	470	1	36	37	61
94	HPMBQ32	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	104	1351	1	1351	18	18	342	1	23	24	86
95	HPWAN23	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	105	2066	51	2052	270	270	343	1	29	30	537
95	HPWAN23	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	233	2057	1	1954	220	220	471	1	29	30	315



Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
96	HRDFB85	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	106	1705	23	1697	233	233	344	1	21	22	201
97	HRGBR28	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	107	1167	1	557	604	604	345	1	22	23	122
98	HSKGN81	97977 04/04/97 209082 05/29/97	pBluescript	108	1907	151	1432	353	353	346	1	23	24	260
98	HSKGN81	97977 04/04/97 209082 05/29/97	pBluescript	234	2084	335	2084	537	537	472	1	19	20	23
99	HSPA56	97977 04/04/97 209082 05/29/97	pSport1	109	611	1	576	229	229	347	1	25	26	47
100	HE8EU04	209746 04/07/98	Uni-ZAP XR	110	2632	294	2632	337	337	348	1	25	26	333

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of 5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
100	HSXBT86	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	235	2143	53	1096	235	235	473	1			9
101	HSXCS62	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	111	2249	1	1953	90	90	349	1	18	19	199
102	HTEFU09	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	112	2198	228	2158	400	400	350	1			23
103	HTEKM35	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	113	1043	40	1043	320	320	351	1	20	21	142
104	HTGEP89	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	114	703	1	703	285	285	352	1	29	30	94

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
105	HTGEW91	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	115	3684	526	1338	584	584	353	1	24	25	37
106	HTOEY16	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	116	1965	127	1915	202	202	354	1	27	28	38
107	HTPCN79	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	117	503	1	503		1	355	1	7	8	70
108	HTSGM54	97977 04/04/97 209082 05/29/97	pBluescript	118	1071	50	981	29	29	356	1	30	31	227
108	HTSGM54	97977 04/04/97 209082 05/29/97	pBluescript	236	1133	316	1069		423	474	1	12	13	84

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
109	HTSHE40	97977 04/04/97 209082 05/29/97	pBluescript	119	1101	118	956	218	218	357	1	31	32	89
110	HTWAF58	97977 04/04/97 209082 05/29/97	Lambda ZAP II	120	282	1	282	137	137	358	1	25	26	48
111	HTWBY29	97977 04/04/97 209082 05/29/97	pSport1	121	2635	1593	2489	1654	1654	359	1	25	26	55
112	HUKFC71	209007 04/28/97 209083 05/29/97	Lambda ZAP II	122	994	1	932		272	360	1	15	16	221
113	HCE3Q10	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	123	1542	1	1542	143	143	361	1	25	26	63

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
114	HCEVR60	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	124	1390	82	1390	127	127	362	1	32	33	153
115	HDTAW95	209007 04/28/97 209083 05/29/97	pCMVSPORT 2.0	125	1288	412	1288	571	571	363	1			16
116	HE6EL90	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	126	1517	1	1452	243	243	364	1			9
117	HELB29	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	127	1073	198	1073		776	365	1			13
118	HERAH36	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	128	300	155	300	202	202	366	1			17

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
119	HFXBW82	209007 04/28/97 209083 05/29/97	Lambda ZAP II	129	1275	1	1275	56	56	367	1	23	24	61
120	HHPD20	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	130	472	51	472	243	243	368	1			32
121	HIBED17	209007 04/28/97 209083 05/29/97	Other	131	1950	284	1927	395	395	369	1	72	73	245
122	HLTER03	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	132	990	1	990	78	78	370	1	22	23	34
123	HOABL56	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	133	1720	565	1720	660	660	371	1	18	19	21

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
124	HPMCJ92	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	134	705	28	705	106	106	372	1	28	29	98
125	HPWAZ95	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	135	323	1	323	88	88	373	1	27	28	78
126	HRGBR18	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	136	582	1	582		16	374	1	17	18	30
127	HSUBW09	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	137	1021	1	1021	153	153	375	1	32	33	56
128	HUKCO64	209007 04/28/97 209083 05/29/97	Lambda ZAP II	138	1777	1	1339	198	198	376	1	23	24	63

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
129	H6EAA53	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	139	643	303	643	306	306	377	1	14	15	38
130	HAGAI11	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	140	1220	1	1220	567	567	378	1	50	51	98
131	HAGAO39	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	141	721	1	721	415	415	379	1			14
132	HALSK07	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	142	1468	125	1468	210	210	380	1	29	30	33
133	HALSQ59	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	143	300	4	300	101	101	381	1	22	23	66
134	HAIBP89	209877 05/18/98	Uni-ZAP XR	144	2243	173	2243	311	311	382	1	27	28	317



Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
134	HBGCB91	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	237	1025	409	1025	624	475	1	20	21	25
135	HBM TD81	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	145	1082	163	1082	357	383	1			30
136	HBXGK12	209008 04/28/97 209084 05/29/97	ZAP Express	146	4313	1153	4313	1313	384	1	18	19	42
137	HFKFJ07	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	147	1183	1	1183	149	385	1	41	42	254
138	HCQA140	209008 04/28/97 209084 05/29/97	Lambda ZAP II	148	734	1	734	285	386	1			19

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
139	HCWHZ24	209008 04/28/97 209084 05/29/97	ZAP Express	149	1405	1	1405	108	108	387	1	34	35	63
140	HE2GT20	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	150	2890	1178	2890	1178	1178	388	1	31	32	39
141	HE8EY43	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	151	2399	1181	2399	1265	1265	389	1	30	31	34
142	HFCEB37	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	152	802	352	802		487	390	1			10
143	HFTCT67	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	153	461	24	461	145	145	391	1	37	38	63

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
144	HGLAM46	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	154	2388	818	2388	648	648	392	1			18
145	HHGBR15	209008 04/28/97 209084 05/29/97	Lambda ZAP II	155	642	322	642	369	369	393	1	41	42	43
146	HJAAU36	209008 04/28/97 209084 05/29/97	pBluescript SK-	156	1251	583	1251		933	394	1	16	17	16
147	HUSIT49	209008 04/28/97 209084 05/29/97	pSport1	157	2127	247	2127	383	383	395	1	47	48	83
148	HKLAB16	209008 04/28/97 209084 05/29/97	Lambda ZAP II	158	1625	817	1625	1012	1012	396	1	18	19	20

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
149	HLMMU76	209008 04/28/97 209084 05/29/97	Lambda ZAP II	159	1687	1307	1687	1296	1296	397	1	28	29	28
150	HMSKQ35	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	160	1842	172	1463	319	319	398	1	30	31	33
151	HNHED86	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	161	770	1	770	30	30	399	1	31	32	46
152	HNHEJ88	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	162	519	1	519	242	242	400	1	17	18	24
153	HNHFQ63	209008 04/28/97 209084 05/29/97	Uni-ZAP XR	163	753	1	753	164	164	401	1	17	18	67
154	HOECU83	209009 04/28/97	Uni-ZAP XR	164	1893	1	1211	1637	1637	402	1	28	29	85

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
154	HOECU83	209009 04/28/97	Uni-ZAP XR	238	1400	189	1400		508	476	1	22	23	33
155	HPTRC15	209009 04/28/97	pBluescript	165	2153	594	2153	57	57	403	1	26	27	82
156	HSKCP69	209009 04/28/97	Uni-ZAP XR	166	1251	219	1120	49	49	404	1	27	28	286
156	HSKCP69	209009 04/28/97	Uni-ZAP XR	239	1250	223	1250	393	393	477	1	32	33	171
157	H6EAE26	209009 04/28/97	Uni-ZAP XR	167	882	48	882	155	155	405	1	33	34	153
158	HAGBX03	209009 04/28/97	Uni-ZAP XR	168	1208	1	1208	290	290	406	1	20	21	37
159	HAGDQ47	209009 04/28/97	Uni-ZAP XR	169	1258	1	1258	44	44	407	1	22	23	60
159	HAGDQ47	209009 04/28/97	Uni-ZAP XR	240	1307	1	1307	44	44	478	1	22	23	60
160	HAICP19	209009 04/28/97	Uni-ZAP XR	170	1624	89	1483	128	128	408	1	18	19	446
161	HAUAE83	209009 04/28/97	Uni-ZAP XR	171	2003	889	2003	957	957	409	1	29	30	64
162	HBHAD12	209009 04/28/97	Uni-ZAP XR	172	786	1	786		176	410	1	17	18	23

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
163	HBM TY28	209009 04/28/97	Uni-ZAP XR	173	1758	962	1758	1184	1184	411	1	27	28	34
164	HBM VP04	209009 04/28/97	Uni-ZAP XR	174	1369	29	557	947	947	412	1	33	34	41
164	HBM VP04	209009 04/28/97	Uni-ZAP XR	241	888	330	862		546	479	1			2
165	HCDD B78	209009 04/28/97	Uni-ZAP XR	175	2379	750	2379	901	901	413	1	18	19	24
166	HCEQA68	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	176	1348	1	1348	12	12	414	1	28	29	78
167	HCEZS40	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	177	1502	178	1502	388	388	415	1	31	32	51
168	HCFNF11	209010 04/28/97 209085 05/29/97	pSport1	178	1637	26	1607	152	152	416	1	44	45	257

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
169	HCRBL20	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	179	2911	1103	2858	192	192	417	1	32	33	424
169	HCRBL20	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	242	1811	20	1811	93	93	480	1	36	37	95
170	HCUBL62	209010 04/28/97 209085 05/29/97	ZAP Express	180	519	1	519	57	57	418	1	28	29	32
171	HDSAP81	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	181	968	320	968	476	476	419	1	27	28	79
172	HE2CT29	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	182	1128	1	1128	111	111	420	1	26	27	94

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
173	HE8MG65	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	183	2276	48	2276	88	88	421	1	37	38	257
173	HE8MG65	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	243	2271	56	2232	79	79	481	1	43	44	170
174	HE9FB42	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	184	3374	86	1705	277	277	422	1	40	41	704
174	HE9FB42	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	244	2500	76	1693	518	518	482	1	1	2	623
175	HEMAM41	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	185	1337	60	1328	175	175	423	1	39	40	190



Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
175	HEMAM41	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	245	1338	33	1327	175	175	483	1	32	33	91
176	HEMCV19	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	186	941	33	931	79	79	424	1	23	24	178
177	HEMDX17	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	187	678	1	678	131	131	425	1	21	22	40
177	HEMDX17	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	246	654	1	654	137	137	484	1			12
178	HETAR54	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	188	1848	454	1848	948	948	426	1	14	15	232

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
179	HETBX14	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	189	1292	303	1292	207	207	427	1	18	19	250
179	HETBX14	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	247	1146	157	1146	74	485	1	14	15	53	
180	HFGAB48	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	190	906	156	906	628	428	1	23	24	58	
181	HFKFI40	209010 04/28/97 209085 05/29/97	Uni-ZAP XR	191	1941	120	1002	213	213	429	1	18	19	218
182	HFXHN68	209010 04/28/97 209085 05/29/97	Lambda ZAP II	192	2118	777	2118	966	430	1	23	24	50	
183	HGBFO79	209011 04/28/97	Uni-ZAP XR	193	1538	259	1538	273	431	1	23	24	49	

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
184	HGLAM56	209011 04/28/97	Uni-ZAP XR	194	1098	68	1098		185	432	1	28	29	69
185	HHLBA89	209011 04/28/97	pBluescript SK-	195	1001	1	1001	324	324	433	1	25	26	39
186	HHPDW05	209011 04/28/97	Uni-ZAP XR	196	1458	1	1458	254	254	434	1	17	18	104
186	HHPDW05	209011 04/28/97	Uni-ZAP XR	248	1443	1	1443	246	246	486	1	21	22	21
187	HHPD37	209011 04/28/97	pBluescript	197	1282	66	1282	171	171	435	1	19	20	37
188	HHPD70	209011 04/28/97	pBluescript	198	951	26	951		162	436	1	16	17	34
189	HHSK25	209011 04/28/97	Uni-ZAP XR	199	1740	1390	1740	1534	1534	437	1	19	20	31
190	HIASB53	209011 04/28/97	pBluescript	200	1707	401	1195	652	652	438	1	26	27	126
191	HJABZ65	209011 04/28/97	pBluescript SK-	201	779	1	779	23	23	439	1	26	27	68
192	HJPBB39	209011 04/28/97	Uni-ZAP XR	202	1617	188	1605	182	182	440	1	28	29	91
193	HLHKS94	209011 04/28/97	pBluescript	203	1974	1	1794	112	112	441	1	26	27	379

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
194	HLHTC70	209011 04/28/97	pBluescript	204	1057	229	1057	365	365	442	1	23	24	22
195	HLMIW92	209011 04/28/97	Lambda ZAP II	205	721	1	721	244	244	443	1	25	26	46
196	HLTCY93	209011 04/28/97	Uni-ZAP XR	206	2465	988	2465	387	387	444	1	27	28	214
197	HLTDB65	209011 04/28/97	Uni-ZAP XR	207	1480	1	1480		371	445	1	15	16	143
198	HMSHM43	209011 04/28/97	Uni-ZAP XR	208	872	1	872	35	35	446	1	18	19	36
199	HMSHQ24	209011 04/28/97	Uni-ZAP XR	209	1779	16	1779	148	148	447	1	24	25	36
200	HNFAH08	209011 04/28/97	Uni-ZAP XR	210	2110	592	2110	611	611	448	1	18	19	191
201	HNGAO10	209011 04/28/97	Uni-ZAP XR	211	938	1	938	107	107	449	1	27	28	30
202	HNGBE45	209011 04/28/97	Uni-ZAP XR	212	1551	1	1551	114	114	450	1	21	22	100
203	HNHAZ16	209011 04/28/97	Uni-ZAP XR	213	997	1	997	202	202	451	1	24	25	36
204	HNHCM59	209011 04/28/97	Uni-ZAP XR	214	1496	1	1132		165	452	1	28	29	41

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
205	HOSFM22	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	215	1308	501	1308	1081	1081	453	1	46	47	48
206	HPHAC88	97977 04/04/97 209082 05/29/97	Uni-ZAP XR	216	1705	384	1705	549	549	454	1	23	24	24
207	HCDEO95	209007 04/28/97 209083 05/29/97	Uni-ZAP XR	217	999	608	999	273	273	455	1	22	23	54

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also

5 hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

10 Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the

15 actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the

20 generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods.

25 The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

30 The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.



The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

### Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of

predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

5 In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis  
10 of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence  
15 shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present  
20 invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of  
25 directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present  
30 invention.

### **Polynucleotide and Polypeptide Variants**

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

5       The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many  
10       regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the  
15       nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).  
20       Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%,  
25       95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95%  
30       "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each

100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then

subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference

sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal

residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are

included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.



Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or

fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

## 5      **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

10      In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, 15 and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger 20 or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

25      Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 30 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this

context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed  
 5 herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the  
 10 cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,  
 15 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

20 Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-  
 25 60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also  
 30 preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and

alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

- 5 Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments.

- 10 Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

- 15 Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a
- 20 polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

- 25 The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

- For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using
- 30 techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using

colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, *Microbiol. Rev.* 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

### **Epitopes and Antibodies**

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses

polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,

that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., *Cell* 37:767-778 (1984); Sutcliffe et al., *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle et al., *J. Gen. Virol.* 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an



immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example, polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof, resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opinion Biotechnol.* 8:724-33

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(1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### **Antibodies**

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin

molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be

excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog,

5 ortholog, or homolog of a polypeptide of the present invention are included.

Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present

10 invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using

15 methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation

25 constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the

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epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes

5 antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand

10 binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described

15 supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent

20 ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but

25 do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists

30 for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.

5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of

numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

5 The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal  
10 antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and  
15 potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be  
20 produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not  
25 limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma  
30 technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an

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immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage

gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol.*

Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into

mouse embryonic stem cells in addition to the human heavy and light chain genes.

The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human

immunoglobulin loci by homologous recombination. In particular, homozygous

5 deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention.

10 Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation.

Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA,

15 IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European  
20 Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar  
25 to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et  
30 al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using

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techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### *Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library

generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entirety), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino

acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038-1041 (1988)).

#### *Methods of Producing Antibodies*

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a

polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as



bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems

5 infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,

10 BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole

15 recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2

20 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the

25 generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic*

30 *Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such

fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt,

which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 5 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. 10 (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

15 The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in 20 culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second 25 vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic 30 free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any

combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entirety).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to

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identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred  
5   embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags  
10   useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used  
15   diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent  
20   materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No.  
25   4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone,  
30   fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin,

and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),



granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### *Immunophenotyping*

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific

epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### *Assays For Antibody Binding*

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel *et al.*, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A

and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes

the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

#### *Therapeutic Uses*

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any

one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention,

including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

### *Gene Therapy*

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other

desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific  
5   embodiments, the expressed antibody molecule is a single chain antibody;  
alternatively, the nucleic acid sequences include sequences encoding both the heavy  
and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or  
10   indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be  
15   accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun;  
20   Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target  
25   cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT  
30   Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination

(Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.



Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages,

neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

#### *Demonstration of Therapeutic or Prophylactic Activity*

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

*Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment;

this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by

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the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

#### *Diagnosis and Imaging*

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, et al., *J. Cell Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods



including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce  
5 diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of  
10 Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the  
15 labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by  
20 repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label  
25 used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

30 In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with

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a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is  
5 detected in a patient using magnetic resonance imaging (MRI).

### *Kits*

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified  
10 antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present  
15 invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

20 In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically  
25 immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide  
30 antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may

also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound

recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### **Fusion Proteins**

5 Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target  
10 cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur  
15 through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell  
20 or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and  
25 specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins  
30 consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)

Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

### **Vectors, Host Cells, and Protein Production**

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated

or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency  
 5 from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the  
 10 polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate  
 15 high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia*  
 20 *pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in  
 25 the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows  
 30 expression and secretion of a protein of the invention by virtue of the strong *AOX1*



promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entirety).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and

Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid  
 5 analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine,  
 10 norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

15 The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not  
 20 limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of  
 25 N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and  
 30 isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as

increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG

to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated

5 polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for

10 therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid,

15 glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

20 One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be

25 performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be

30 accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions,

substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (*i.e.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional  
5   embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as,  
10   for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion  
15   protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( *e.g.*, that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one  
20   instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (*i.e.*, naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the  
25   heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, *e.g.*, US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as  
30   described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for

example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous



polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers  
5 of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues

10 located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers  
15 containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its  
20 entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent  
25 Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse  
30 orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described

herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by  
5 reference in its entirety).

### **Uses of the Polynucleotides**

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes  
10 known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present  
15 invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of  
20 somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per  
25 day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends  
30 Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see

5 Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

10 The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-

15 492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage

20 analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated

25 with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural

30 alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide

and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or

estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the

preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al.,

"The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)).

Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by  
 5 insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelman et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelman et al., supra) Indeed, the human counterparts of the oncogenes involved in  
 10 some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelman et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International  
 15 Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580;  
 20 Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit  
 25 proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca  
 30 Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the

polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a



unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of the Polypeptides**

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics

of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which  
5 involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high  
10 amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the  
15 development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different  
20 polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a  
25 desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce  
30 overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

### **Gene Therapy Methods**

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired

5 polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye,

10 gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph

15 fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated

20 cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20

25 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

30 The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or

bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of

DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

5 Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC),  
 10 dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC),  
 15 dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2  
 20 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available  
 25 to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology, 101:512-527 (1983),  
 30 which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated.



SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors

may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

5       The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by  
10       reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

15       The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

20       In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional  
25       mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science , 252:431-434 (1991);  
30       Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

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Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell, 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor

Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses.

- 5 Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

- 10 Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 15 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

- Polynucleotide constructs are made, using standard techniques known in the 20 art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous 25 polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

- The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same 30 restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the

amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle

accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppository solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium

- 5 phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries.

Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA, 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a

polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

#### **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention, and/or treatment of diseases and/or disorders associated with the following systems.

#### **Immune Activity**

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis,

producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in the "FEATURES OF PROTEIN" section for each gene.

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired immunodeficiencies.

Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked

immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type),

Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID),

common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.



In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof.

5        Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare  
10 lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

15        In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using polypeptides or polynucleotides of the invention, and/or agonists or  
20 antagonists thereof, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic  
25 alymphoplasia-aplasia, immunodeficiency with thymoma, severe congenital leukopenia, dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented,  
30 diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, diagnosed and/or prognosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura), autoimmunocytopenia, Goodpasture's syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, neuritis, uveitis ophthalmia, polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary

inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention.

In preferred embodiments, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, prognosing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, Polynucleotides, polypeptides, antibodies, and/or

agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists or antagonists thereof, may be used to treat, prevent, diagnose and/or prognose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain

injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are

5 characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

Because inflammation is a fundamental defense mechanism, inflammatory

10 disorders can effect virtually any tissue of the body. Accordingly, polynucleotides, polypeptides, and antibodies of the invention, as well as agonists or antagonists thereof, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis,

15 chorditis, cochlitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis,

20 peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose,

25 prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues.

Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or

30 antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD. In specific embodiments, polypeptides,

antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

5           In other embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

10           Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an  
15           existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

20           In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance tumor-specific immune responses.

25           In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art.

30           In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In

another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meissneria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat

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infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

10 In one embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an  
15 immune response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell responsiveness to pathogens.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an activator of T cells.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent that elevates the immune status of an individual prior to their receipt of  
25 immunosuppressive therapies.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to induce higher affinity antibodies.

30 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to increase serum immunoglobulin concentrations.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to accelerate recovery of immunocompromised individuals.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among aged populations and/or neonates.

10 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, 15 compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

25 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists 30 or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious

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mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used in the pretreatment of bone marrow samples prior to transplant.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a gene-based

therapy for genetically inherited disorders resulting in immuno-  
incompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as a means of  
5 activating monocytes/macrophages to defend against parasitic diseases that effect  
monocytes such as Leishmania.

In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as a means of  
regulating secreted cytokines that are elicited by polypeptides of the invention.

10 In another embodiment, polypeptides, antibodies, polynucleotides and/or  
agonists or antagonists of the present invention are used in one or more of the  
applications described herein, as they may apply to veterinary medicine.

In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as a means of  
15 blocking various aspects of immune responses to foreign agents or self. Examples of  
diseases or conditions in which blocking of certain aspects of immune responses may  
be desired include autoimmune disorders such as lupus, and arthritis, as well as  
immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and  
diseases/disorders associated with pathogens.

20 In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as a therapy for  
preventing the B cell proliferation and Ig secretion associated with autoimmune  
diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus  
and multiple sclerosis.

25 In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as an inhibitor of B  
and/or T cell migration in endothelial cells. This activity disrupts tissue architecture  
or cognate responses and is useful, for example in disrupting immune responses, and  
blocking sepsis.

30 In another specific embodiment, polypeptides, antibodies, polynucleotides  
and/or agonists or antagonists of the present invention are used as a therapy for  
chronic hypergammaglobulinemia evident in such diseases as monoclonal

gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed for instance  
5 to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

10 The polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit  
15 complement mediated cell lysis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may also be employed for  
20 treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed to treat adult  
25 respiratory distress syndrome (ARDS).

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the repair of  
30 vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to diagnose, prognose, treat, and/or prevent a disorder characterized

by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with polynucleotides or polypeptides, and/or agonists of the present invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled "Hyperproliferative Disorders" elsewhere herein.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

5 In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, 10 such as, for example, an individual who has undergone a partial or complete splenectomy.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes or soluble forms of the polypeptides of the present invention (e.g., Fc fusion protein; see, e.g., Example 9). Agonists of the 15 invention include, for example, binding or stimulatory antibodies, and soluble forms of the polypeptides (e.g., Fc fusion proteins; see, e.g., Example 9). polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

20 In another embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention are administered to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of 25 producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741). Administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention to such animals is useful for the generation of 30 monoclonal antibodies against the polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present invention in an organ system listed above.

**Blood-Related Disorders**

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hemostatic (the stopping of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes.

Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the

polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to



prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in the "FEATURES OF PROTEIN" section for each gene.

5           The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes,  
10   lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of anemias and leukopenias described below. Alternatively, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the  
15   present invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for  
20   example eosinophilia.

          The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to prevent, treat, or diagnose blood dyscrasia.

          Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may  
25   be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the polynucleotides, polypeptides, antibodies, and/or agonists or  
30   antagonists of the present invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious

anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hemolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias arising from drug treatments such as anemias associated with methyldopa, dapsone, and/or sulfadruugs. Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating thalassemias, including, but not limited to major and minor forms of alpha-thalassemia and beta-thalassemia.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thromboasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophelias such as hemophelia A or Factor VII deficiency and Christmas disease or Factor IX deficiency, Hereditary

Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

The effect of the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention on the clotting time of blood may be monitored  
5 using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in  
10 a specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction accompanying kidney failure, leukemia, multiple myeloma, cirrhosis of the liver, and systemic lupus erythematosus as well as platelet dysfunction associated with drug  
15 treatments, including treatment with aspirin, ticlopidine, nonsteroidal anti-inflammatory drugs (used for arthritis, pain, and sprains), and penicillin in high doses.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders characterized by or  
20 associated with increased or decreased numbers of white blood cells. Leukopenia occurs when the number of white blood cells decreases below normal. Leukopenias include, but are not limited to, neutropenia and lymphocytopenia. An increase in the number of white blood cells compared to normal is known as leukocytosis. The body generates increased numbers of white blood cells during infection. Thus, leukocytosis  
25 may simply be a normal physiological parameter that reflects infection. Alternatively, leukocytosis may be an indicator of injury or other disease such as cancer.

Leukocytoses, include but are not limited to, eosinophilia, and accumulations of macrophages. In specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in  
30 diagnosing, prognosing, preventing, and/or treating leukopenia. In other specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or

antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukocytosis.

Leukopenia may be a generalized decreased in all types of white blood cells, or may be a specific depletion of particular types of white blood cells. Thus, in specific embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating decreases in neutrophil numbers, known as neutropenia. Neutropenias that may be diagnosed, prognosed, prevented, and/or treated by the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, infantile genetic agranulocytosis, familial neutropenia, cyclic neutropenia, neutropenias resulting from or associated with dietary deficiencies (e.g., vitamin B 12 deficiency or folic acid deficiency), neutropenias resulting from or associated with drug treatments (e.g., antibiotic regimens such as penicillin treatment, sulfonamide treatment, anticoagulant treatment, anticonvulsant drugs, anti-thyroid drugs, and cancer chemotherapy), and neutropenias resulting from increased neutrophil destruction that may occur in association with some bacterial or viral infections, allergic disorders, autoimmune diseases, conditions in which an individual has an enlarged spleen (e.g., Felty syndrome, malaria and sarcoidosis), and some drug treatment regimens.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating lymphocytopenias (decreased numbers of B and/or T lymphocytes), including, but not limited lymphocytopenias resulting from or associated with stress, drug treatments (e.g., drug treatment with corticosteroids, cancer chemotherapies, and/or radiation therapies), AIDS infection and/or other diseases such as, for example, cancer, rheumatoid arthritis, systemic lupus erythematosus, chronic infections, some viral infections and/or hereditary disorders (e.g., DiGeorge syndrome, Wiskott-Aldrich Syndrome, severe combined immunodeficiency, ataxia telangiectasia).

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with macrophage numbers and/or

macrophage function including, but not limited to, Gaucher's disease, Niemann-Pick disease, Letterer-Siwe disease and Hand-Schuller-Christian disease.

In another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing,

- 5 prognosing, preventing, and/or treating diseases and disorders associated with eosinophil numbers and/or eosinophil function including, but not limited to, idiopathic hypereosinophilic syndrome, eosinophilia-myalgia syndrome, and Hand-Schuller-Christian disease.

- 10 In yet another embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing, prognosing, preventing, and/or treating leukemias and lymphomas including, but not limited to, acute lymphocytic (lymphoblastic) leukemia (ALL), acute myeloid (myelocytic, myelogenous, myeloblastic, or myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., B cell leukemias, T cell leukemias, Sezary syndrome, and
- 15 Hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, or granulocytic) leukemia, Hodgkin's lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, and mycosis fungoides.

- In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in diagnosing,
- 20 prognosing, preventing, and/or treating diseases and disorders of plasma cells including, but not limited to, plasma cell dyscrasias, monoclonal gammaopathies, monoclonal gammopathies of undetermined significance, multiple myeloma, macroglobulinemia, Waldenstrom's macroglobulinemia, cryoglobulinemia, and Raynaud's phenomenon.

- 25 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing myeloproliferative disorders, including but not limited to, polycythemia vera, relative polycythemia, secondary polycythemia, myelofibrosis, acute myelofibrosis, agnogenic myelod metaplasia, thrombocythemia, (including both
- 30 primary and seconday thrombocythemia) and chronic myelocytic leukemia.

In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as a treatment prior to surgery, to increase blood cell production.

5 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to enhance the migration, phagocytosis, superoxide production, antibody dependent cellular cytotoxicity of neutrophils, eosinophils and macrophages.

10 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to stem cells pheresis. In another specific embodiment, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase the number of stem cells in circulation prior to platelet pheresis.

15 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful as an agent to increase cytokine production.

20 In other embodiments, the polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in preventing, diagnosing, and/or treating primary hematopoietic disorders.

### **Hyperproliferative Disorders**

25 In certain embodiments, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

30 For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune

response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue

- Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related
- 5 Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular
- 10 Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant
- 15 Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and
- 20 Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer,
- 25 Ovarian Germ Cell Tumor, Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma,
- 30 Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive



Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal

5 Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In another preferred embodiment, polynucleotides or polypeptides, or agonists or antagonists of the present invention are used to diagnose, prognose, prevent, and/or

10 treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders described above. Such uses are indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal

15 growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W. B. Saunders Co., Philadelphia, pp. 68-79.)

Hyperplasia is a form of controlled cell proliferation, involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Hyperplastic disorders which can be diagnosed, prognosed, prevented, and/or treated

20 with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, angiofollicular mediastinal lymph node hyperplasia, angiolymphoid hyperplasia with eosinophilia, atypical melanocytic hyperplasia, basal cell hyperplasia, benign giant lymph node hyperplasia, cementum hyperplasia, congenital adrenal hyperplasia, congenital sebaceous hyperplasia, cystic

25 hyperplasia, cystic hyperplasia of the breast, denture hyperplasia, ductal hyperplasia, endometrial hyperplasia, fibromuscular hyperplasia, focal epithelial hyperplasia, gingival hyperplasia, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, intravascular papillary endothelial hyperplasia, nodular hyperplasia of prostate, nodular regenerative hyperplasia, pseudoepitheliomatous hyperplasia,

30 senile sebaceous hyperplasia, and verrucous hyperplasia.

Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplastic disorders

which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, agnogenic myeloid metaplasia, apocrine metaplasia, atypical metaplasia, autoparenchymatous metaplasia, connective tissue metaplasia, epithelial metaplasia, intestinal metaplasia, metaplastic anemia, metaplastic ossification, metaplastic polyps, myeloid metaplasia, primary myeloid metaplasia, secondary myeloid metaplasia, squamous metaplasia, squamous metaplasia of amnion, and symptomatic myeloid metaplasia.

Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation. Dysplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, anhidrotic ectodermal dysplasia, anterofacial dysplasia, asphyxiating thoracic dysplasia, atriодigital dysplasia, bronchopulmonary dysplasia, cerebral dysplasia, cervical dysplasia, chondroectodermal dysplasia, cleidocranial dysplasia, congenital ectodermal dysplasia, craniodiaphysial dysplasia, craniocarpotarsal dysplasia, craniometaphysial dysplasia, dentin dysplasia, diaphysial dysplasia, ectodermal dysplasia, enamel dysplasia, encephalo-ophthalmic dysplasia, dysplasia epiphysialis hemimelia, dysplasia epiphysialis multiplex, dysplasia epiphysialis punctata, epithelial dysplasia, faciodigitogenital dysplasia, familial fibrous dysplasia of jaws, familial white folded dysplasia, fibromuscular dysplasia, fibrous dysplasia of bone, florid osseous dysplasia, hereditary renal-retinal dysplasia, hidrotic ectodermal dysplasia, hypohidrotic ectodermal dysplasia, lymphopenic thymic dysplasia, mammary dysplasia, mandibulofacial dysplasia, metaphysial dysplasia, Mondini dysplasia, monostotic fibrous dysplasia, mucoepithelial dysplasia, multiple epiphysial dysplasia, oculoauriculovertebral dysplasia, oculodentodigital dysplasia, oculovertеbral dysplasia, odontogenic dysplasia, ophthalmomandibulomelic dysplasia, periapical cemental dysplasia, polyostotic fibrous dysplasia,

pseudoachondroplastic spondyloepiphyseal dysplasia, retinal dysplasia, septo-optic dysplasia, spondyloepiphyseal dysplasia, and ventriculoradial dysplasia.

Additional pre-neoplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention (including

5 polynucleotides, polypeptides, agonists or antagonists) include, but are not limited to, benign dysproliferative disorders (e.g., benign tumors, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, and esophageal dysplasia), leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis.

In another embodiment, a polypeptide of the invention, or polynucleotides,  
10 antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in the "FEATURES OF PROTEIN" section for each gene.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or  
15 agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat cancers and neoplasms, including, but not limited to those described herein. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein,  
20 may be used to treat acute myelogenous leukemia.

Additionally, polynucleotides, polypeptides, and/or agonists or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the  
25 inhibition of apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer,  
30 intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and

ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

In preferred embodiments, polynucleotides, polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma,

Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or

agonists or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include, but are not limited to, neoplasms located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Another preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells.

10 In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably  
15 an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other  
20 polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the  
25 present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the  
30 destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By

"biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present



invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than  $5 \times 10^{-6} \text{M}$ ,  $10^{-6} \text{M}$ ,  $5 \times 10^{-7} \text{M}$ ,  $10^{-7} \text{M}$ ,  $5 \times 10^{-8} \text{M}$ ,  $10^{-8} \text{M}$ ,  $5 \times 10^{-9} \text{M}$ ,  $10^{-9} \text{M}$ ,  $5 \times 10^{-10} \text{M}$ ,  $10^{-10} \text{M}$ ,  $5 \times 10^{-11} \text{M}$ ,  $10^{-11} \text{M}$ ,  $5 \times 10^{-12} \text{M}$ ,  $10^{-12} \text{M}$ ,  $5 \times 10^{-13} \text{M}$ ,  $10^{-13} \text{M}$ ,  $5 \times 10^{-14} \text{M}$ ,  $10^{-14} \text{M}$ ,  $5 \times 10^{-15} \text{M}$ , and  $10^{-15} \text{M}$ .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, anti-inflammatory proteins

(See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

5 Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4  
10 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions  
15 containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic  
20 and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and  
25 immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

### **Renal Disorders**

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the  
30 present invention, may be used to treat, prevent, diagnose, and/or prognose disorders of the renal system. Renal disorders which can be diagnosed, prognosed, prevented,

and/or treated with compositions of the invention include, but are not limited to, kidney failure, nephritis, blood vessel disorders of kidney, metabolic and congenital kidney disorders, urinary disorders of the kidney, autoimmune disorders, sclerosis and necrosis, electrolyte imbalance, and kidney cancers.

- 5           Kidney diseases which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, acute kidney failure, chronic kidney failure, atheroembolic renal failure, end-stage renal disease, inflammatory diseases of the kidney (e.g., acute glomerulonephritis, postinfectious glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome,
- 10   membranous glomerulonephritis, familial nephrotic syndrome, membranoproliferative glomerulonephritis I and II, mesangial proliferative glomerulonephritis, chronic glomerulonephritis, acute tubulointerstitial nephritis, chronic tubulointerstitial nephritis, acute post-streptococcal glomerulonephritis (PSGN), pyelonephritis, lupus nephritis, chronic nephritis, interstitial nephritis, and post-streptococcal
- 15   glomerulonephritis), blood vessel disorders of the kidneys (e.g., kidney infarction, atheroembolic kidney disease, cortical necrosis, malignant nephrosclerosis, renal vein thrombosis, renal underperfusion, renal retinopathy, renal ischemia-reperfusion, renal artery embolism, and renal artery stenosis), and kidney disorders resulting from urinary tract disease (e.g., pyelonephritis, hydronephrosis, urolithiasis (renal lithiasis,
- 20   nephrolithiasis), reflux nephropathy, urinary tract infections, urinary retention, and acute or chronic unilateral obstructive uropathy.)

- In addition, compositions of the invention can be used to diagnose, prognose, prevent, and/or treat metabolic and congenital disorders of the kidney (e.g., uremia, renal amyloidosis, renal osteodystrophy, renal tubular acidosis, renal glycosuria,
- 25   nephrogenic diabetes insipidus, cystinuria, Fanconi's syndrome, renal fibrocystic osteosis (renal rickets), Hartnup disease, Bartter's syndrome, Liddle's syndrome, polycystic kidney disease, medullary cystic disease, medullary sponge kidney, Alport's syndrome, nail-patella syndrome, congenital nephrotic syndrome, CRUSH syndrome, horseshoe kidney, diabetic nephropathy, nephrogenic diabetes insipidus,
- 30   analgesic nephropathy, kidney stones, and membranous nephropathy), and autoimmune disorders of the kidney (e.g., systemic lupus erythematosus (SLE),

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Goodpasture syndrome, IgA nephropathy, and IgM mesangial proliferative glomerulonephritis).

Compositions of the invention can also be used to diagnose, prognose, prevent, and/or treat sclerotic or necrotic disorders of the kidney (e.g.,

- 5 glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), necrotizing glomerulonephritis, and renal papillary necrosis), cancers of the kidney (e.g., nephroma, hypernephroma, nephroblastoma, renal cell cancer, transitional cell cancer, renal adenocarcinoma, squamous cell cancer, and Wilm's tumor), and electrolyte imbalances (e.g., nephrocalcinosis, pyuria, edema,
- 10 hydronephritis, proteinuria, hyponatremia, hypernatremia, hypokalemia, hyperkalemia, hypocalcemia, hypercalcemia, hypophosphatemia, and hyperphosphatemia).

- Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous
- 15 injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more
- 20 detail below. Methods of delivering polynucleotides are described in more detail herein.

### **Cardiovascular Disorders**

- Polynucleotides or polypeptides, or agonists or antagonists of the present
- 25 invention, may be used to treat, prevent, diagnose, and/or prognose cardiovascular disorders, including, but not limited to, peripheral artery disease, such as limb ischemia.

- Cardiovascular disorders include, but are not limited to, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral
- 30 arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include, but are not limited to, aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia,

patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects,

- 5 Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include, but are not limited to, heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac  
 10 edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease,  
 15 ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

- Arrhythmias include, but are not limited to, sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine  
 20 Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia,  
 25 Torsades de Pointes, and ventricular tachycardia.

- Heart valve diseases include, but are not limited to, aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia,  
 30 tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include, but are not limited to, alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular

stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include, but are not limited to, coronary disease, such as  
 5 angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema,  
 10 aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's  
 15 disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include, but are not limited to, dissecting aneurysms, false  
 20 aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include, but are not limited to, arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery  
 25 occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include, but are not limited to, carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis,  
 30 Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia

(including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include, but are not limited to, air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include, but are not limited to, coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemic disorders include, but are not limited to, cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes, but is not limited to, aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

### **Respiratory Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be used to treat, prevent, diagnose, and/or prognose diseases and/or disorders of the respiratory system.

Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis, juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's

nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomonas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal infections in people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus spp.*; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia spp.*), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis,



desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

#### **Anti-Angiogenesis Activity**

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech. J.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present

invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*,

5 Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists  
10 may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder,  
15 thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and  
20 Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a  
25 catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These  
30 disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy,

retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists of the invention are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a mucoadhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion,

but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or

agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated, prevented, diagnosed, and/or prognosed with the the polynucleotides, polypeptides, agonists and/or antagonists of the invention include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochelle minalia quintosa*), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or antagonists may also be used in controlling menstruation or administered as either a

peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch  
5 granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal  
10 surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic  
15 compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-  
20 angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the  
25 site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly  
30 preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum



(VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

5 A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence  
10 of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem.  
15 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987);  
20 Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

### 25 **Diseases at the Cellular Level**

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include  
30 cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma,

lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma,

craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, diagnosed, and/or prognosed using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or

antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentum graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis.

- 5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat
- 10 gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or
- 15 antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect
- 20 the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associated with the under expression.

- 25 Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke
- 30 inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as

agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

5 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins  
10 known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists  
15 of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

#### 20 **Neural Activity and Neurological Diseases**

The polynucleotides, polypeptides and agonists or antagonists of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system disorders that can be treated with the compositions of the invention (e.g., polypeptides, polynucleotides,  
25 and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the methods of the invention, include but are not limited to, the  
30 following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or

ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In one embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of hypoxia. In a further preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated

with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

In another preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, 10:3507-15 (1990); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, 70:65-82 (1980), or Brown *et al.*, *Ann. Rev.*



*Neurosci.*, 4:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Further, polypeptides or polynucleotides of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including polynucleotides, polypeptides, and agonists or antagonists) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, polypeptides, polynucleotides and/or agonists or antagonists of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell

leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention

include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral

5 toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, and cerebral malaria.

- 10 Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis, Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as
- 15 Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as
- 20 Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.

- Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include central nervous system neoplasms such as brain neoplasms that include
- 25 cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sclerolus which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell
- 30 leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis,

transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon-Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman

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such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

#### **Endocrine Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose disorders and/or diseases related to hormone imbalance, and/or disorders or diseases of the endocrine system.

Hormones secreted by the glands of the endocrine system control physical growth, sexual function, metabolism, and other functions. Disorders may be classified in two ways: disturbances in the production of hormones, and the inability of tissues to respond to hormones. The etiology of these hormone imbalance or endocrine system diseases, disorders or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy, injury or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular disease or disorder related to the endocrine system and/or hormone imbalance.

Endocrine system and/or hormone imbalance and/or diseases encompass disorders of uterine motility including, but not limited to: complications with

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pregnancy and labor (e.g., pre-term labor, post-term pregnancy, spontaneous abortion, and slow or stopped labor); and disorders and/or diseases of the menstrual cycle (e.g., dysmenorrhea and endometriosis).

Endocrine system and/or hormone imbalance disorders and/or diseases include

- 5 disorders and/or diseases of the pancreas, such as, for example, diabetes mellitus, diabetes insipidus, congenital pancreatic agenesis, pheochromocytoma--islet cell tumor syndrome; disorders and/or diseases of the adrenal glands such as, for example, Addison's Disease, corticosteroid deficiency, virilizing disease, hirsutism, Cushing's Syndrome, hyperaldosteronism, pheochromocytoma; disorders and/or diseases of the
- 10 pituitary gland, such as, for example, hyperpituitarism, hypopituitarism, pituitary dwarfism, pituitary adenoma, panhypopituitarism, acromegaly, gigantism; disorders and/or diseases of the thyroid, including but not limited to, hyperthyroidism, hypothyroidism, Plummer's disease, Graves' disease (toxic diffuse goiter), toxic nodular goiter, thyroiditis (Hashimoto's thyroiditis, subacute granulomatous
- 15 thyroiditis, and silent lymphocytic thyroiditis), Pendred's syndrome, myxedema, cretinism, thyrotoxicosis, thyroid hormone coupling defect, thymic aplasia, Hurthle cell tumours of the thyroid, thyroid cancer, thyroid carcinoma, Medullary thyroid carcinoma; disorders and/or diseases of the parathyroid, such as, for example, hyperparathyroidism, hypoparathyroidism; disorders and/or diseases of the
- 20 hypothalamus.

- 25 In specific embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists of those polypeptides (including antibodies) as well as fragments and variants of those polynucleotides, polypeptides, agonists and antagonists, may be used to diagnose, prognose, treat, prevent, or ameliorate diseases and disorders associated with aberrant glucose metabolism or glucose uptake into cells.

- 30 In a specific embodiment, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists and/or antagonists thereof may be used to diagnose, prognose, treat, prevent, and/or ameliorate type I diabetes mellitus (insulin dependent diabetes mellitus, IDDM).

In another embodiment, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists and/or antagonists thereof may be used to



diagnose, prognose, treat, prevent, and/or ameliorate type II diabetes mellitus (insulin resistant diabetes mellitus).

Additionally, in other embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or antagonists thereof (especially neutralizing or antagonistic antibodies) may be used to diagnose, prognose, treat, prevent, and/or ameliorate conditions associated with (type I or type II) diabetes mellitus, including, but not limited to, diabetic ketoacidosis, diabetic coma, nonketotic hyperglycemic-hyperosmolar coma, seizures, mental confusion, drowsiness, cardiovascular disease (e.g., heart disease, atherosclerosis, microvascular disease, hypertension, stroke, and other diseases and disorders as described in the "Cardiovascular Disorders" section), dyslipidemia, kidney disease (e.g., renal failure, nephropathy other diseases and disorders as described in the "Renal Disorders" section), nerve damage, neuropathy, vision impairment (e.g., diabetic retinopathy and blindness), ulcers and impaired wound healing, infections (e.g., infectious diseases and disorders as described in the "Infectious Diseases" section, especially of the urinary tract and skin), carpal tunnel syndrome and Dupuytren's contracture.

In other embodiments, the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to regulate the animal's weight. In specific embodiments the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to control the animal's weight by modulating a biochemical pathway involving insulin. In still other embodiments the polynucleotides and/or polypeptides corresponding to this gene and/or agonists or antagonists thereof are administered to an animal, preferably a mammal, and most preferably a human, in order to control the animal's weight by modulating a biochemical pathway involving insulin-like growth factor.

In addition, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases of the testes or ovaries, including cancer. Other disorders and/or diseases of the testes or ovaries further include, for example, ovarian cancer, polycystic ovary syndrome, Klinefelter's syndrome, vanishing testes syndrome (bilateral anorchia), congenital absence of Leydig's cells,

cryptorchidism, Noonan's syndrome, myotonic dystrophy, capillary haemangioma of the testis (benign), neoplasias of the testis and neo-testis.

Moreover, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases such as, for example,

- 5 polyglandular deficiency syndromes, pheochromocytoma, neuroblastoma, multiple Endocrine neoplasia, and disorders and/or cancers of endocrine tissues.

In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose, prognose, prevent, and/or treat endocrine diseases and/or disorders  
10 associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in the "FEATURES OF PROTEIN" section for each gene.

#### **Reproductive System Disorders**

- 15 The polynucleotides or polypeptides, or agonists or antagonists of the invention may be used for the diagnosis, treatment, or prevention of diseases and/or disorders of the reproductive system. Reproductive system disorders that can be treated by the compositions of the invention, include, but are not limited to, reproductive system injuries, infections, neoplastic disorders, congenital defects, and  
20 diseases or disorders which result in infertility, complications with pregnancy, labor, or parturition, and postpartum difficulties.

Reproductive system disorders and/or diseases include diseases and/or disorders of the testes, including testicular atrophy, testicular feminization, cryptorchism (unilateral and bilateral), anorchia, ectopic testis, epididymitis and  
25 orchitis (typically resulting from infections such as, for example, gonorrhea, mumps, tuberculosis, and syphilis), testicular torsion, vasitis nodosa, germ cell tumors (e.g., seminomas, embryonal cell carcinomas, teratocarcinomas, choriocarcinomas, yolk sac tumors, and teratomas), stromal tumors (e.g., Leydig cell tumors), hydrocele, hematocele, varicocele, spermatocele, inguinal hernia, and disorders of sperm  
30 production (e.g., immotile cilia syndrome, aspermia, asthenozoospermia, azoospermia, oligospermia, and teratozoospermia).

Reproductive system disorders also include disorders of the prostate gland, such as acute non-bacterial prostatitis, chronic non-bacterial prostatitis, acute bacterial prostatitis, chronic bacterial prostatitis, prostatodystonia, prostatosis, granulomatous prostatitis, malacoplakia, benign prostatic hypertrophy or hyperplasia, and prostate  
 5 neoplastic disorders, including adenocarcinomas, transitional cell carcinomas, ductal carcinomas, and squamous cell carcinomas.

Additionally, the compositions of the invention may be useful in the diagnosis, treatment, and/or prevention of disorders or diseases of the penis and urethra, including inflammatory disorders, such as balanoposthitis, balanitis xerotica  
 10 obliterans, phimosis, paraphimosis, syphilis, herpes simplex virus, gonorrhea, non-gonococcal urethritis, chlamydia, mycoplasma, trichomonas, HIV, AIDS, Reiter's syndrome, condyloma acuminatum, condyloma latum, and pearly penile papules; urethral abnormalities, such as hypospadias, epispadias, and phimosis; premalignant lesions, including Erythroplasia of Queyrat, Bowen's disease, Bowenoid paplosis,  
 15 giant condyloma of Buscke-Lowenstein, and verrucous carcinoma; penile cancers, including squamous cell carcinomas, carcinoma in situ, verrucous carcinoma, and disseminated penile carcinoma; urethral neoplastic disorders, including penile urethral carcinoma, bulbomembranous urethral carcinoma, and prostatic urethral carcinoma; and erectile disorders, such as priapism, Peyronie's disease, erectile dysfunction, and  
 20 impotence.

Moreover, diseases and/or disorders of the vas deferens include vasculitis and CBAVD (congenital bilateral absence of the vas deferens); additionally, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or  
 25 disorders of the seminal vesicles, including hydatid disease, congenital chloride diarrhea, and polycystic kidney disease.

Other disorders and/or diseases of the male reproductive system include, for example, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes mellitus, cystic fibrosis, Kartagener's syndrome, high fever, multiple sclerosis, and  
 30 gynecomastia.

Further, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of

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diseases and/or disorders of the vagina and vulva, including bacterial vaginosis, candida vaginitis, herpes simplex virus, chancroid, granuloma inguinale, lymphogranuloma venereum, scabies, human papillomavirus, vaginal trauma, vulvar trauma, adenositis, chlamydia vaginitis, gonorrhea, trichomonas vaginitis, condyloma acuminatum, syphilis, molluscum contagiosum, atrophic vaginitis, Paget's disease, lichen sclerosus, lichen planus, vulvodynia, toxic shock syndrome, vaginismus, vulvovaginitis, vulvar vestibulitis, and neoplastic disorders, such as squamous cell hyperplasia, clear cell carcinoma, basal cell carcinoma, melanomas, cancer of Bartholin's gland, and vulvar intraepithelial neoplasia.

Disorders and/or diseases of the uterus include dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding (e.g., due to aberrant hormonal signals), and neoplastic disorders, such as adenocarcinomas, leiomyosarcomas, and sarcomas. Additionally, the polypeptides, polynucleotides, or agonists or antagonists of the invention may be useful as a marker or detector of, as well as in the diagnosis, treatment, and/or prevention of congenital uterine abnormalities, such as bicornuate uterus, septate uterus, simple unicornuate uterus, unicornuate uterus with a noncavitary rudimentary horn, unicornuate uterus with a non-communicating cavitary rudimentary horn, unicornuate uterus with a communicating cavitary horn, arcuate uterus, uterine didelphys, and T-shaped uterus.

Ovarian diseases and/or disorders include anovulation, polycystic ovary syndrome (Stein-Leventhal syndrome), ovarian cysts, ovarian hypofunction, ovarian insensitivity to gonadotropins, ovarian overproduction of androgens, right ovarian vein syndrome, amenorrhea, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, Sertoli-Leydig tumors, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, and Ovarian Krukenberg tumors).

Cervical diseases and/or disorders include cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, and cervical neoplasms (including, for example,

cervical carcinoma, squamous metaplasia, squamous cell carcinoma, adenosquamous cell neoplasia, and columnar cell neoplasia).

Additionally, diseases and/or disorders of the reproductive system include disorders and/or diseases of pregnancy, including miscarriage and stillbirth, such as early abortion, late abortion, spontaneous abortion, induced abortion, therapeutic abortion, threatened abortion, missed abortion, incomplete abortion, complete abortion, habitual abortion, missed abortion, and septic abortion; ectopic pregnancy, anemia, Rh incompatibility, vaginal bleeding during pregnancy, gestational diabetes, intrauterine growth retardation, polyhydramnios, HELLP syndrome, abruptio placentae, placenta previa, hyperemesis, preeclampsia, eclampsia, herpes gestationis, and urticaria of pregnancy. Additionally, the polynucleotides, polypeptides, and agonists or antagonists of the present invention may be used in the diagnosis, treatment, and/or prevention of diseases that can complicate pregnancy, including heart disease, heart failure, rheumatic heart disease, congenital heart disease, mitral valve prolapse, high blood pressure, anemia, kidney disease, infectious disease (e.g., rubella, cytomegalovirus, toxoplasmosis, infectious hepatitis, chlamydia, HIV, AIDS, and genital herpes), diabetes mellitus, Graves' disease, thyroiditis, hypothyroidism, Hashimoto's thyroiditis, chronic active hepatitis, cirrhosis of the liver, primary biliary cirrhosis, asthma, systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenic purpura, appendicitis, ovarian cysts, gallbladder disorders, and obstruction of the intestine.

Complications associated with labor and parturition include premature rupture of the membranes, pre-term labor, post-term pregnancy, postmaturity, labor that progresses too slowly, fetal distress (e.g., abnormal heart rate (fetal or maternal), breathing problems, and abnormal fetal position), shoulder dystocia, prolapsed umbilical cord, amniotic fluid embolism, and aberrant uterine bleeding.

Further, diseases and/or disorders of the postdelivery period, including endometritis, myometritis, parametritis, peritonitis, pelvic thrombophlebitis, pulmonary embolism, endotoxemia, pyelonephritis, saphenous thrombophlebitis, mastitis, cystitis, postpartum hemorrhage, and inverted uterus.

Other disorders and/or diseases of the female reproductive system that may be diagnosed, treated, and/or prevented by the polynucleotides, polypeptides, and

agonists or antagonists of the present invention include, for example, Turner's syndrome, pseudohermaphroditism, premenstrual syndrome, pelvic inflammatory disease, pelvic congestion (vascular engorgement), frigidity, anorgasmia, dyspareunia, ruptured fallopian tube, and Mittelschmerz.

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### **Infectious Disease**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

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Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papilloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic

fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific

embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial and fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following Gram-

15 Negative and Gram-positive bacteria, bacterial families, and fungi: Actinomyces  
(e.g., Norcardia), Acinetobacter, *Cryptococcus neoformans*, Aspergillus, Bacillaceae  
(e.g., *Bacillus anthracis*), Bacteroides (e.g., *Bacteroides fragilis*), Blastomycosis,  
Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucella, Candidia, Campylobacter,  
Chlamydia, Clostridium (e.g., *Clostridium botulinum*, *Clostridium difficile*,  
20 *Clostridium perfringens*, *Clostridium tetani*), Coccidioides, Corynebacterium (e.g.,  
*Corynebacterium diphtheriae*), Cryptococcus, Dermatocycoses, *E. coli* (e.g.,  
Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (e.g.,  
*Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (e.g.,  
*Salmonella typhi*, *Salmonella enteritidis*, *Salmonella typhi*), Serratia, Yersinia,  
25 Shigella), Erysipelothrix, Haemophilus (e.g., *Haemophilus influenza* type B),  
Helicobacter, Legionella (e.g., *Legionella pneumophila*), Leptospira, Listeria (e.g.,  
*Listeria monocytogenes*), Mycoplasma, Mycobacterium (e.g., *Mycobacterium leprae*  
and *Mycobacterium tuberculosis*), Vibrio (e.g., *Vibrio cholerae*), Neisseriaceae (e.g.,  
*Neisseria gonorrhea*, *Neisseria meningitidis*), Pasteurellaceae, Proteus, Pseudomonas  
30 (e.g., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (e.g., Treponema spp.,  
Leptospira spp., Borrelia spp.), Shigella spp., Staphylococcus (e.g., *Staphylococcus*  
*aureus*), Meningioccus, Pneumococcus and Streptococcus (e.g., *Streptococcus*

*pneumoniae* and Groups A, B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye infections (e.g., conjunctivitis), uveitis, tuberculosis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., meningitis types A and B), chlamydia, syphilis, diphtheria, leprosy, brucellosis, peptic ulcers, anthrax, spontaneous abortions, birth defects, pneumonia, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory diseases, candidiasis, paratuberculosis, tuberculosis, lupus, botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections, noscomial infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Schistosoma, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium virax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be



used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage.

Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of

non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

#### **Gastrointestinal Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat, prevent, diagnose, and/or prognose gastrointestinal disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowel lymphoma)), and ulcers, such as peptic ulcers.

Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adeoncarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers, polyps of the stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum, hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, bubphrenic abscess,).

Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata*, *Echinococcus granulosus*, *Diphyllobothrium spp.*, and *T. solium*).

Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolenticular degeneration, hepatomegaly, hepatopulmonary syndrome, hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile

hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, 5 hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Kaposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]], peliosis hepatis, erythrohepatic porphyria, hepatic 10 porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and 15 glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic fibrosis, cyst (pancreatic pseudocyst, pancreatic fistula, insufficiency)).

Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, 20 acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

Diseases and/or disorders of the large intestine include antibiotic-associated colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous 25 adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon [Hirschsprung disease, toxic megacolon]; sigmoid diseases [proctocolitis, sigmoid neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases 30 (ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease (ulcerative colitis, Crohn's disease), intestinal atresia, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis,

amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction, impacted feces, intestinal pseudo-obstruction [cecal volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jeghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowel syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing enteropathies (intestinal lymphagiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse, rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer, Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia, gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and hemorrhagic colitis.

Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms), esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, atresia, cyst, diverticulum (e.g., Zenker's diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome),

stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)),  
 5 hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal diseases (appendicitis, cecal neoplasms)).

### **Chemotaxis**

10 Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then  
 15 fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the  
 20 number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as  
 25 agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

### **Binding Activity**

30 A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The

binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

5 Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable  
10 of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane  
15 containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving  
20 competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate  
25 compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The  
30 antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

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Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., *Current Protocols in Immun.*, 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*, *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al.*, *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R.



*Biotechniques* 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by

5 homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be

10 recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor

15 (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

20 Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

25 Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and  $^3\text{[H]}$  thymidine under cell culture conditions where the fibroblast cell would normally

30 proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the

uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

5 In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following  
10 interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

15 All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from  
20 suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of  
25 identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

### **Targeted Delivery**

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

5 As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

20 By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of

benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

5           **Drug Screening**

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected  
10   compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in  
15   such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation  
20   of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the  
25   present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a  
30   particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present

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invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

#### **Polypeptides of the Invention Binding Peptides and Other Molecules**

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind polypeptides of the invention, and the polypeptide of the invention binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of: contacting a polypeptide of the invention with a plurality of molecules; and identifying a molecule that binds the polypeptide of the invention.

The step of contacting the polypeptide of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptide of the invention on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptide of the invention. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized polypeptide of the

invention. The molecules having a selective affinity for the polypeptide of the invention can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptide of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptide of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptide of the invention and the individual clone. Prior to contacting the polypeptide of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for a polypeptide of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the polypeptide of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound polypeptide of the invention, or alternatively, unbound polypeptides, from a mixture of the polypeptide of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction.

Such a wash step may be particularly desirable when the polypeptide of the invention or the plurality of polypeptides is bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind to a polypeptide of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, *Science* 251:767-773; Houghten et al., 1991, *Nature* 354:84-86; Lam et al., 1991, *Nature* 354:82-84; Medynski, 1994, *Bio/Technology* 12:709-710; Gallop et al., 1994, *J. Medicinal Chemistry* 37(9):1233-1251; Ohlmeyer et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:10922-10926; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422-11426; Houghten et al., 1992, *Biotechniques* 13:412; Jayawickreme et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:1614-1618; Salmon et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, *Proc. Natl. Acad. Sci. USA* 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, *Science* 249:386-390; Devlin et al., 1990, *Science*, 249:404-406; Christian, R. B., et al., 1992, *J. Mol. Biol.* 227:711-718; Lenstra, 1992, *J. Immunol. Meth.* 152:149-157; Kay et al., 1993, *Gene* 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, *Proc. Natl. Acad. Sci. USA* 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is

great. For example, Ecker and Crooke, 1995, *Bio/Technology* 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992; *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds a polypeptide of the invention can be carried out by contacting the library members with a polypeptide of the invention immobilized on a solid phase and harvesting those



library members that bind to the polypeptide of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

5 In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to a polypeptide of the invention.

10 Where the polypeptide of the invention binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

15 Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine.

20 Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

25 As mentioned above, in the case of a polypeptide of the invention binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a polypeptide of the invention binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

30 The selected polypeptide of the invention binding polypeptide can be obtained by chemical synthesis or recombinant expression.

### **Antisense And Ribozyme (Antagonists)**

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited  
 5 clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA  
 10 or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300  
 15 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These  
 20 experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is  
 25 heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA  
 30 oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense

RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA.

Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981)), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980)), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981)), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents. (See, *e.g.*, Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-O-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of

the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

#### **Other Activities**

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide

may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to



modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to treat weight disorders, including but not limited to, obesity, cachexia, wasting disease, anorexia, and bulimia.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

#### **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the  
5 ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide  
10 sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human  
15 cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological  
20 sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained  
25 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences  
30 comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence

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selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human

cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a

sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained  
5 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

10 Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at  
15 least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

20 Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules  
25 in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in  
30 Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

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Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.



Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-

human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

- 5 In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 2, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3
- 10 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 2. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby
- 15 incorporated by reference in their entirety.

TABLE 2

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
1	HLHDS67	11	396448	T84556, R77553, H77877, H96723, N22894, N24112, N25474, N31281, N31410, N31809, N42470, N58904, N59834, N67726, W03552, W15430, W78090, W79576, W94783, W95299, AA112608, AA126875, AA127799, AA133859, AA169532, AA169601
2	HLHDZ58	12	396869	R44557, R44557, H15251, H16568
10	HOUBE18	20	407070	T97913, R21634, R47833, R49975, R54690, R55015, R55153, R62188, R64576, R80153, R80154, R81484, R81724, H13709, H13762, H49782, N33449, N34466, N42422, N42873, N50673, N53663, N73029, W44598, W73379, W73403, AA088385
11	HOUDL69	21	396821	T98572, T98573, T99692, R46104, R46177, R46104, R46177, R77699, R77698, R81185, R81291, R84758, R84835, N73056, W88438, W89202
12	HPMFI71	22	407378	R53416, R54007, H14084, H45951, H75270, H75382, N27106, N40516, W37083, W37084, W72173
15	HPTBB03	25	399928	T58022, T86930, R11711, T83207, T86107,

				T96449, R17686, R36056, R36058, R49138, R49140, R53540, R53651, R49138, R73230, R76352, H06054, H13390, H14662, H17478, H17586, H24833, H29049, H29151, H92319, H92379, N24774, N32793, N42234, N94618, W15347, W31392, W31984, W39439, W95395, W95353, AA088664, AA088803, AA102451, AA130481, AA130482, AA143411, AA143667, AA146597, AA148224, AA148225, AA156280, AA156391, AA158602, AA158959, AA158958, AA158971, AA158970, AA164777
16	HPTWA66	26	614220	R32953, R48005, R52174, R53999, R94185, N58829, N75247, W86429, AA024852, AA024935, AA101581, AA101582, AA121348, AA121367, AA135194, AA135274, AA149607, AA149718, AA181794, AA461476, AA460122
16	HPTWA66	219	408041	T56759, T63654, R48005, R53999, N58829, W86429, AA024852, AA101582, AA121348, AA135194, AA149607
17	HPTWC08	27	396380	T77302, R21500, R35136, R41732, R42882, R49522, R41732, R42882, R49522, H20938, H41732, R85141, R88669, R88670, R88816, R89638, R89643, R90743, R90777, R90782, AA040665, AA127052
18	HRGCZ46	28	400796	T48000, T49441, T62059, T65112, T65179, T92082, T78688, T79315, T83158, T85864, R15724, R17015, R18665, R22674, R45966, R45966, H24497, H27416, H44475, N50917, N94040, W17223, W40134, W92875, W94259, W94444, W94673, W94957, W95142, W95598, W95853, N89726, AA045010, AA081572
19	HSAVU34	29	724060	T52500, T67115, T67116, T90451, R10617, R10618, T82973, H05156, H10930, H10931, H56169, H56385, H66700, H66701, H73933, H74126, N32119, N57071, N59463, N67109, N71110, N74124, N74136, W02046, W05471, W19600, W23443, W24737, W35258, W37178, W57794, W58026, W81529, W81530, AA079135, AA121270, AA121423, AA151481, AA151504, AA220993, AA226857, AA250826, AA252645, AA428383
19	HSAVU34	220	396807	T52500, T67115, T67116, T90451, R10617, R10618, T82973, H05156, H10930, H10931, H56169, H56385, H66700, H66701, H73933, H74126, N32119, N57071, N59463, N67109, N71110, N74124, N74136, W02046, W05471, W19600, W23443, W24737, W35258, W37178, W57794, W58026, W81529, W81530, AA079135, AA121270, AA121423, AA151481, AA151504
20	HSDFW61	30	407496	T55525, R10577, R10576, R11610, T78468, T78545, T95431, R01101, R19400, H55969, H84552, N24342, N26542, N35654, N39425, N48541, N64022, N73360, N78008, N95084, W23486, W67558, W67606, W69403, W73515, W73497, W74493, W79090, N89865, AA015719, AA034158, AA053058, AA053402, AA127181

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22	HSOAJ55	32	829668	T90006, R09378, R09379, R12195, T82827, T84829, R23136, R23137, R23150, R23149, R23901, R23902, R35690, R39919, R49327, R49327, H08646, H08645, H52709, H52986, H67120, H81426, H97481, N20907, N31009, N51872, N51878, N54463, N76574, W37292, W37826, AA052963, AA053019, AA053505, AA129021, AA129020, AA133655, AA133656, AA130953, AA573417, AA746147, AA879142, AA938486, D82776, D82683, W23236, C17493
22	HSOAJ55	221	361281	T90006, R09378, R09379, R12195, T82827, T84829, R23136, R23137, R23150, R23149, R23901, R23902, R35690, R39919, R49327, R49327, H08646, H08645, H52709, H52986, H67120, H81426, H97481, N20907, N31009, N51872, N51878, N54463, N76574, W37292, W37826, AA052963, AA053019, AA053505, AA129021, AA129020, AA133655, AA133656
24	HSXAM05	34	396445	H41055, H86278, H86277, N36167, N49355, N99253, W30680, AA187548
25	HSXAS67	35	396441	R43052, R46024, R54365, R46024, R59349, H29581, AA018134, AA128286, AA165397
26	HTDAF28	36	396835	R32754, R65808
30	HTPBW79	40	581435	T58875, T69236, R12437, R13448, R37325, R37361, N52277, W38735, W72124, AA009696, AA088448, AA181149, AA181148
30	HTSEV09	223	396459	T54203, T58875, T69236, R37325, R37361, R72050, N52277, N59026, N72929, W38735, W72124, W77848, AA009696, AA009415, AA088448, AA088502, AA181149
31	HJPCD40	41	401227	R01078, H47562, H58468, N31052, N92318, N93676, N93667, W24390, W79518, W79405, W86336, W95427, W95554, AA002106, AA005054, AA009752, AA009751, AA022648, AA022637, AA035194, AA143435, AA157417
33	HTWCI46	43	407490	T71107, R07491, R07544, R02367, R02473, R12602, R74032, R74123, R79290, R81173, R81277, R86952, H49320, N54909, AA196897
34	HTXGI75	44	396652	H11517, H61199, H96603, N24777, N28311, N28909, N73009, N73300, W02991, W16442, W23776, W35231, W39312, W79539, W79620, AA026925, AA026924, AA079258, AA079257, AA085612, AA112862, AA143350, AA143349, AA147394, AA147466, AA147465, AA156313
35	HWTBF59	45	740670	T47527, T47528, T89276, T84349, R00444, R00445, R50263, R50726, R51643, R62425, R73541, H05078, H38730, H68907, H68809, H75646, H75453, H75452, H81932, H82027, N32427, N36140, N37025, N42766, N44144, N52649, N56850, N68925, W02115, W03625, W15447, W20382, W32576, W35117, W39632, W44562, W47604, W69467, W69551, W73740, W86128, W86148, W95672, AA024821, AA024927, AA025859, AA025860, AA046830, AA046873, AA126258, AA134986, AA135083, AA150759, AA150682, AA235603, AA236621,

				AA236897, AA464236, AA419070, AA419131, AA428777, AA429067, AA428056
35	HWTBF59	224	361287	T47528, T89276, R00445, R50726, R51643, R62425, R73541, H05078, H38730, H68907, H75646, H75453, H81932, N32427, N36140, N37025, N52649, N56850, N68925, W02115, W15447, W20382, W32576, W39632, W44562, W69551, W73740, W86128, W86148, W95672, AA024821, AA024927, AA025859, AA025860, AA046830, AA126258, AA134986, AA135083, AA150759
36	HADAE74	46	409832	T46989, T46988, T65134, T65203, R17426, R23237, R23312, R42660, H60654, H75862, H75861, N24093, N31388, W56001, W56290, AA047063, AA047064, AA046111, AA046198, AA098961, AA098828, AA182785, AA187777, AA191047
38	HATEF60	48	410124	T64995, R17261, R41876, R68452, R68454, H21498, H98622, N25142, N30676, N33908, N67489, N99057, W30718, AA035240, AA035318, AA043654, AA043655, AA046927, AA046984, AA133159, AA133204, AA131580, AA131629, AA132763, AA132857
39	HBMSN25	49	412010	R34536, R49053, R85085, R87831, R87846, AA199833
40	HCDAR68	50	411482	H24669, AA055330, AA055927
41	HCE3J79	51	409610	T66611, T81694, R15985, R44533, R50983, R52279, R52280, R54333, R46724, R44533, R61702, R63949, R64049, R72902, R73540, H42845, H43343, H43397, H44022, H44570, H44569, H59659, H74011, H75604, N53399, W60971, W61218, AA024497, AA024619, AA132738, AA173154, AA188369, AA237026
42	HMDAN54	52	411318	T78112, R19702, R37848, R44258, R44258
43	HCECA49	53	409543	T48789, T48790, T52689, T52690, T54143, T57627, T60334, T63169, T64611, T68165, T73770, R09683, R05784, R05870, R23705, R24243, R25436, R26263, R26661, R31482, R33617, R52663, R55790, R64491, R65588, R66756, R74348, R74447, R77767, R77861, H24648, H24647, H25483, H30170, H42201, H61272, H74187, H73366, H84457, H96852, H97161, N21258, N24067, N25891, N32256, N35943, N39665, N59887, N74237, N75946, N77028, N91815, N94382, W16791, W37991, W42625, W42503, W42504, W45097, W46997, W47010, W47011, W47035, W58226, W60191, W74239, AA011342, AA053421, AA053142, AA069730, AA069687, AA071401, AA079362, AA088476, AA088867, AA099339, AA098900, AA099401, AA099509, AA099626, AA100481, AA111899, AA112344, AA128689, AA130068, AA130069, AA133988, AA134388, AA130699, AA131164, AA135908, AA143614, AA148147, AA151655, AA151855, AA150148, AA152217, AA150454, AA156656, AA156942, AA158064,

				AA158065, AA160927, AA167640, AA173558, AA173723, AA188571, AA188806, AA190996, AA191121
44	HCEEC15	54	409527	R69381, R69382
45	HCESF40	55	616396	R13472
45	HCESF40	225	411082	R13472, R37382, H49570, N55573
46	HCFMV39	56	410579	R91923, R92247
48	HCNAP62	58	411042	H21798, AA149965
49	HCRAF32	59	409522	AA194845
53	HE2AV74	63	411019	R33678, R35656, R37491, R56683, H14646, H61361, H62387, AA131445, AA131558
54	HE2AY71	64	396403	T67822, T67974, T73185, T67263, T67264, T91311, T84892, T85089, R22026, R22079, R23310, R25617, R31409, R33081, R33171, R33622, R33733, R48174, R48558, R48654, R66621, R73320, R73769, R74211, R74309, R82212, R82268, R82548, H03235, H03847, H19922, H46963, H46964, H47061, H47135, R91968, R94507, R94914, R94997, R98001, R99462, R99463, R99524, R99525, R99727, H48529, H48701, H53102, H54578, H57772, H59377, H61224, H61728, H62607, H65068, H65067, H66144, H66346, H66396, H66561, H67023, H67024, H67947, H68317, H68316, H70368, H75943, H78688, H78690, H78771, H78772, H78871, H79256, H79366, H85283, H94696, H98582, H98844, H99986, N20645, N24174, N25213, N26654, N29017, N30434, N33496, N36055, N39390, N39804, N42325, N43023, N43886, N44135, N44905, N55434, N58351, N58498, N59566, N68659, N72973, N73562, N74053, N74661, N75286, N76807, N77719, N78566, N80677, N93248, N93543, N98928, N98927, W00492, W00999, W01748, W04563, W04661, W05686, W07160, W07727, W17036, W20423, W20166, W20366, W21351, W23644, W31155, W31425, W33072, W35181, W37772, W37773, W39698, W45053, W45703, W44350, W46232, W46853, W55895, W55894, W57879, W57878, W72198, W73477, W73549, W92689, W94064, W94065, W94685, W95191, W95291, N89780, N89860, N90540, N91134, AA026253, AA026254, AA026166, AA029566, AA034238, AA037765, AA046097, AA053931, AA062822, AA082444, AA085263, AA085327, AA128794, AA128795, AA147331, AA191231, AA195440
55	HE2GS36	65	779386	N31459, AA027911, AA045421
55	HE2GS36	226	411492	R41228, H09131, H09953, N25344, N52068, AA027855, AA045315
56	HE2OF09	66	371407	N68961, W00660, W46419, W48762, W49781
57	HE6EU50	67	411998	R10241, R10723, R10745, W86987, AA069424, AA069425
58	HE9HU17	68	411183	T50250, R08461, R08467, R14041, R17411, R33516, R42644, R42644, H01172, H01257, H12436, H12435, H22405, H22406, H46453,

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				H46994, H99758, N28477, N39148, N45470, N46559, N47595, N66302, N70145, N93238, N98322, N98731, W19310, W25095, W31169, W44544, W44408, W57885, W57884, W93786, W93787, W94846, W94847, AA026285, AA026286, AA059054, AA148611, AA148612, AA151931, AA160184, AA160185
60	HEBBW11	70	684293	R12706, R16450, R78468, H04564, H13151, H19832, H19862, H79071, N30846, N92451, W20305, W31335, W87448, W87547, N90683, AA055051, AA055130, AA101604, AA101605, AA262932, AA425185
60	HEBBW11	227	396426	R12706, R78468, H04564, H13151, H19832, H19862, H79071, N30846, N30852, N41379, N92451, W20305, W87448, W87547, N90683, AA044232, AA044371, AA055051, AA101604, AA101605
61	HELDY74	71	410281	T66450, R15824, R51635, W72803
62	HEMAE80	72	409495	T71556, T90634, T82005, T83161, H57113, H61567, AA233071
63	HFEB A88	73	411999	T97504, R01753, H52246, N26214, N50118, N64701, N94589, W23796, W60801, W60932, AA004342, AA063605
64	HFGAB89	74	408358	T89093, R60840, H16750, H51569, H51939
65	HFVHY45	75	410115	N68821
67	HGBBQ69	77	409617	R05775, R05861, R79705, R79706, H14866, H17904, H39588, H40018, H64593, H64594, H64595, H64596, H64613, H64614, H64628, H65957, H65958, H65968, H65967, H66164, H66165, H66166, H66167, H66172, H66173, H66188, H66189, N22434, N59533, N62574, N78274, W61276, W61277, W94640, W92528, AA011621, AA011622, AA040070, AA040101
70	HHFHR32	80	411470	R14689, H04469, H04548, H53694, N26986, N40108, N62880, N79387, W49508, W49509, W55970, W63587, W67453, AA232777, AA233859
71	HHGCN69	81	409956	R71890, R71889, H37817, H37868, N66068, N95661
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73	HHFPD63	83	410143	T75205, R45275, R51873, R54265, R45275, H14061, H14062, H14273, H17115, H17220, H18846, H18847, R85199, R87978, R90826, W73916, W77979, AA169431
76	HKIXL73	86	410511	H19088, H20031, H20111, H46758, H46843, H87138, W70265, W75969
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88	HOSBZ55	98	410145	R70745, H20568
89	HOSDI92	99	617570	R94013, H84608, H98837, N33140, W02553, AA004952, AA429052
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94	HPMBQ32	104	410014	R50088, N75569
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96	HRDFB85	106	411020	R12121, T96099, R05961, R05962, R36883, R48403, R50075, R50076, H13937, H27324, H27350, H44304, H93341, H93844, N72688, W02467, W21446, W74492, W79089, AA149303, AA149402, AA149417, AA149738, AA157596, AA157892
97	HRGBR28	107	410144	T74132, R19091, H16341, H16424, R87393, W74106, AA120808, AA160124
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108	HTSGM54	118	792952	T57851, T82405, R10508, T81626, R14860, N64170, AA114906, AA114905, AA233797, AA233828
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109	HTSHE40	119	411287	R49564, R49564, H72036, W90622
111	HTWBY29	121	410175	T59381, R19528, R43882, R43882, R55664, R55665, H17451, H17555, R88491, R90802, R90803, AA019030, AA021487, AA080905,

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113	HCE3Q10	123	412333	R12129, R15338, R36062, H08308, H14720, H40798, H38530, R88252, R88963, N45514
114	HCEVR60	124	414534	T94052, R63094, R63141, W72684, W73520, W73503, W77790, AA075563, AA075558
115	HDTAW95	125	412472	R46762, R46857
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123	HOABL56	133	413244	R79757, R79756, R92799, R95927, H54516, H83042, N20295, N26162, N27565, N55348, N62316, N77354, N79565, W16550, AA017055
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128	HUKCO64	138	413200	T90943, T79172, T79255, T84324, T85824, T95309, T95390, T99391, R30896, R60293, H58319, H58709, H72088, H72189, H73940, H79782, H79816, H79875, H79910, AA043890, AA045424, AA171926
129	H6EAA53	139	103314	T71026, T71027, T71089, T74115, T74491, T92559, T92631, R31026, R31516, R36638, R47741, R50388, R56704, R79276, R82645, H15896, H16001, H19629, H19628, H19840, H21086, H21123, H21218, H24606, H25286, H25326, H30481, H41893, H41894, H37793, H45153, H45281, H45351, R94255, R94615
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134	HBGCB91	237	371337	T69855, R08029, R08078, H08339, H24045, H42902, H42973, H58361, H58750, H80028, H94211, N70685, N99825, W42711, W42904, W57667, W60487, W60773, AA009753, AA135410
135	HBMTD81	145	410544	R21916, R22565, R99043, AA046203, AA046283, AA055141, AA173411, AA173467
136	HBXGK12	146	415649	T55067, R05951, R76538, R76945, R77034, R79544, R79545, H00668, H61203, H62107, N74280, N77879, W04380, W05836, W07303, AA026385, AA026375, AA047358, AA055622, AA112556, AA159861, AA255749
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141	HE8EY43	151	407475	R74382, R74394, H24509, H89226, N22621, W37881, W37943, W76005, AA215347
142	HFCEB37	152	411345	H06701
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147	HUSIT49	157	421065	T66884, R54992, R55445, H19850, H21231, H22646, H22647, H27769, H27834, H42917, H42918, H43624, H44676, R88710, R90960, R92816, R96930, R96986, R98590, R98589, H60171, H95774, H96129, N54424, N58406, AA129135, AA129134, AA176131, AA195034, AA262891
148	HKLAB16	158	419037	R02500, R32757, R37842, R70640, R82407, W32933, W35369, N90561, AA026880,

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154	HOECU83	164	831917	R34106, R34105, AA166983, AA224458, AA531249, AA588629, C21057
154	HOECU83	238	419012	R34106, R34105, AA224458
155	HPTRC15	165	418375	T90946, T85832, R15053, R60917, R61036, R68361, H05094, H05556, H06465, H10224, H10280, H10972, H10973, H22893, N28604, AA011623, AA011624, AA016231, AA026059, AA166886
156	HSKCP69	166	702021	R09234, R09346, R06914, R06965, H68486, H75419, N67047, W00859, AA029670, AA044243, AA044324, AA148822, AA150422
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157	H6EAE26	167	422804	AA182585, AA243086
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161	HAUAE83	171	422695	T47437, T47436, T47523, T48820, T48821, T53678, T53679, T54444, T54498, T60151, T60211, T63582, T64428, T65689, T65699, T92720, T92800, T74745, T90117, T82456, T82942, T83431, T84078, R19785, R23160, R24260, R24366, R33337, R35278, R36040, R36975, R49121, R50949, R52419, R53809, R53853, R49121, R56655, R56823, R58965, R59021, R63366, R63415, R64167, R64282, R66836, R66884, R67802, R67803, R67933, R67969, R75720, R78064, R80262, R80377, R81338, R81590, H01186, H01282, H08184, H08284, H08404, H08405, H29026, H45836, R97102, R97149, H50658, H50748, H56041, H56118, H65070, H68501, H70503, H88218, H88217, H93598, H93618, N20946, N23947, N27815, N31848, N40220, N51513, N53182, N66179, N66807, N66808, N69755, N98422, N99170, W03608, W38501, W39785, W45318, W46310, W46309, W47477, W47478, W58724, W60790, W60789, W84314, W84341, W94553, W92626, AA022581, AA022582, AA026348, AA026576, AA027051, AA033709, AA034334, AA046827, AA046826, AA045549, AA045550, AA127720, AA127775, AA143073, AA143133, AA150844, AA151016, AA192781, AA192782
163	HBMTY28	173	422688	T54996, T55162, T81957, H40448, H40449, R96511, R96556, H59080, H60352, N58089, N76050, W04455, AA005161, AA004218, AA011395, AA011432, AA116050
164	HBMVP04	174	812281	H82435, H82698, N53899, W04955
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165	HCDDDB78	175	422696	T80138, R05721, R05722, R40720, R51388, R40720, R60772, H77587, H91710, H91811, N52332, N62896, N75102, W01336, W24829,

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167	HCEZS40	177	422714	R12037, R18992, R44878, R44878, H56172, H56388, H58079, H79475, H97586, N20466, N25493, N28755, N50120, N62820, W01355, W74545, W74486, W93543, AA128184, AA126379
168	HCFNF11	178	422712	H80152, AA010492, AA167414, AA167418, AA167415, AA167426, AA167425, AA167419, AA171736, AA172019
169	HCRBL20	179	744946	T89241, H88386, H88454, H88386, N46536, N63060, W93935, W93936, AA075562, AA075557, AA180173, AA180147, AA194932, AA194931, AA194884, AA195588, AA213530, AA243504, AA243357, AA422037
169	HCRBL20	242	422383	T89241, H88386, H88454, H88386, N46536, N63060, W93935, W93936, AA075562, AA075557, AA180147, AA194932, AA194931, AA194884, AA195588, AA213530, AA243504, AA243357
171	HDSAP81	181	422719	N39609, AA011604
172	HE2CT29	182	420020	N74326
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175	HEMAM41	185	741647	R40658, R40658, N62855
175	HEMAM41	245	419870	R40658, R40658, N62855
176	HEMCV19	186	423219	R39576, R39644, R55519, R55520, H25585,

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178	HETAR54	188	422765	R22877, R78124, H86507, N34893, N95529, W20289, W24342, W32533, W32670, N90669, AA019416, AA019318, AA026402, AA027311, AA037586, AA054647, AA252682
179	HETBX14	189	806447	W60282
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180	HFGAB48	190	422777	R42520, R42520, N64660, N80095
181	HFKFI40	191	423226	T47877, T47937, T51505, T75501, T89199, T85240, T85406, R20055, R28467, R31273, R31879, R76266, H03224, H04016, H16963, H30109, N53759, N58780, N62962, N77467, N79865, N81078, W07419, W57548, W68669, W68772
182	HFXHN68	192	422549	T87904, T87997, R10903, R10955, H64853, N63499, N74353, N74407, N94712, W02620, W03115
183	HGBFO79	193	422794	T74861, R54514, R76898, R77063, R79667, R79856, R84453, R98071, H54089, W40292, W46517, W88866, AA203205
184	HGLAM56	194	423223	AA256641, AA256642
187	HHPSD37	197	422805	R44397, R44397, N32549, N41894, AA085999
188	HHPSF70	198	422809	R26136, H08855, H41065, H55993, H80007, H83746, H83889, H88534, H88580, H89097, H89200, N22006, N45466, N45508, N51670, N51854, N54118, N62627, N71208, N78398, AA018235, AA019116, AA131865, AA131952, AA148774, AA148523
189	HHS AK25	199	422813	T92909, T93001, T95997, R61024, H19116, H24430, H24459, R94331, H67161, H68562, H73892, H73918, H74085, H74110, H78993, H81466, H81767, H81766, H82583, H91720, H91821, H99152, N20388, N22843, N24401, N24496, N25453, N28651, N35075, N36359, N43815, W92746, W92869, AA057815
190	HIASB53	200	422811	T68050, R97204, N42257, AA046836, AA047007, AA157267, AA157180, AA186993, AA188308, AA196715
191	HJABZ65	201	419857	N75833, N78710, N91897, W44720, W44764, N90606, AA135838
192	HJPBB39	202	422649	T66427, R15801, R14623, R33639, R45609, R51011, R51118, R45609, R66101, R67704, H17989, H17990, N94819, W17083, W67749, W68029, W74094, W79385, W94890, W92054, AA007307, AA007469, AA054550, AA054558, AA054610, AA054618, AA054521
193	HLHSK94	203	422828	R55809, H83295, N92239, W37154, W38638, N90902, AA017680, AA040604, AA040705
194	HLHTC70	204	422829	R61522, H08810
196	HLTCY93	206	422848	T50389, T50520, T55419, T55495, T55974, T57220, R34591, R34592, R69726, H21148, R85777, R99233, H61311, H62351, H85185, H88299, N23288, N32662, AA005068, AA007333, AA007334, AA036884, AA044715, AA044907,

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				AA045458, AA046500, AA045654, AA115936, AA126775, AA133605, AA133606, AA133980, AA181633, AA182611
197	HLTDB65	207	419864	T88814, T78480, T78565, T84197, T96608, T96718, T96898, T96899, R01674, R02614, R62952, R63004, H01169, H01254, H40397, H53915, H54535, H86324, N23958, N28602, N31859, W17062, W40144, W49624, N89648, AA019070, AA019151, AA134914, AA136931, AA137028, AA148976, AA148977, AA196164, AA196293
199	HMSHQ24	209	422565	R16159, R55052, R59723, R72647, H60244, N33957, N79519, N79654, AA032239, AA033647, AA156948
200	HNFAH08	210	420031	R62825, H69909, H69910, H69910, N25612, N34210, AA056610, AA251839, AA251814
205	HOSFM22	215	412025	T90315, T90402, R23872, R30787, R76172, R77141, R80565, H00726, H01049, H01153, H04603, H04630, H12817, H79113, H82795, H95178, N42743, N68145, N75220, N94419, N98917, W19432, W30766, W31142, W46805, W46923, W48861, W79735, W92123, AA046579, AA046665, AA046966, AA057191, AA127892, AA129011, AA136002, AA136874, AA136903, AA152237, AA152204, AA199930, AA203584
206	HPHAC88	216	411423	R19567, R35876, R35877, R48573, R48673, H41417, R85943
207	HCDEO95	217	371706	H69632, H70475, N66605, AA026327

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Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

## 5      Examples

### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector.

10      Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being  
15      isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited</u>
	<u>Plasmid</u>	
	Lambda Zap	pBluescript (pBS)
20	Uni-Zap XR	pBluescript (pBS)
	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
25	pCMVSPORT 3.0	pCMVSPORT 3.0
	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap  
XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos.  
30      5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Altting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Altting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are



commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

- 5            Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).)
- 10          The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate.
- 15          These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- Alternatively, two primers of 17-20 nucleotides derived from both ends of the
- 20          SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is
- 25          1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and
- 30          the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A<sup>+</sup> RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

**Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X.,  
5 according to the method described in Example 1. (See also, Sambrook.)

**Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by,  
10 among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The  
15 purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to  
20 manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

**Example 4: Chromosomal Mapping of the Polynucleotides**

25 An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C.  
30 Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose

gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

**Example 5: Bacterial Expression of a Polypeptide**

5 A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product  
10 into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning  
15 sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which  
20 expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid  
25 culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to  
30 increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic

agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with

5 high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is

10 eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear

15 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at

20 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains:

25 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

30 DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA

insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

- 5           The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### **Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide  
10   expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield  
15   of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer  
20   (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine  
25   hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with  
30   20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16  $\mu\text{m}$  membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5  $\mu\text{g}$  of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus**

#### **Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40")



is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation

mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

5           Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a  
10   microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is  
15   then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life  
20   Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a  
25   micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

30           To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of

infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of  $^{35}\text{S}$ -methionine and 5 uCi  $^{35}\text{S}$ -cysteine (available from Amersham) are added.

- 5 The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the  
10 produced protein.

#### **Example 8: Expression of a Polypeptide in Mammalian Cells**

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and  
15 signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early  
20 promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109),  
25 pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing  
30 the polynucleotide integrated into a chromosome. The co-transfection with a

selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide.

Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

5        The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

10       Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are  
15       seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in  
20       6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed,  
25       for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### **Example 9: Protein Fusions**

30       The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to

IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create  
 5 chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

10 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion  
 15 can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will  
 20 not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

25 Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCCACC  
 GTGCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCC  
 AAAACCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCG  
 30 TGGTGGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC  
 GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC  
 AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG

GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT  
 CCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA  
 GAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAA  
 CCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCG  
 5 CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCAC  
 GCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCAC  
 CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGA  
 TGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT  
 CCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

10

#### **Example 10: Production of an Antibody from a Polypeptide**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to  
 15 induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are  
 20 monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures  
 25 involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids,  
 30 about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the

present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma  
5 cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is  
10 possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the  
15 polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed  
20 herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use  
25 "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al.,  
30 BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson



et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

### **Example 11: Production Of Secreted Protein For High-Throughput**

#### **5 Screening Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for  
10 a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and  
15 plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

20 The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom  
25 plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

30 Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates

of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130 mg/L CuSO<sub>4</sub>·5H<sub>2</sub>O; 0.050 mg/L of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O; 0.417 mg/L of FeSO<sub>4</sub>·7H<sub>2</sub>O; 311.80 mg/L of KCl; 28.64 mg/L of MgCl<sub>2</sub>; 48.84 mg/L of MgSO<sub>4</sub>; 6995.50 mg/L of NaCl; 2400.0 mg/L of NaHCO<sub>3</sub>; 62.50 mg/L of NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O; 71.02 mg/L of Na<sub>2</sub>HPO<sub>4</sub>; .4320 mg/L of ZnSO<sub>4</sub>·7H<sub>2</sub>O; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed

with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

- 5           The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

- 10           On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

- 15           It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

- 20           One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

- 25           GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with  
30 IL-12. Stat5 was originally called mammary growth factor, but has been found at

higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schindler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

	<u>JAKs</u>					<u>STATS</u>	<u>GAS(elements) or ISRE</u>
	<u>Ligand</u>	<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>		
5	<u>IFN family</u>						
	IFN-a/B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
	IL-10	+	?	?	-	1,3	
10	<u>gp130 family</u>						
	IL-6 (Pleiotrophic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
	IL-11(Pleiotrophic)	?	+	?	?	1,3	
	OnM(Pleiotrophic)	?	+	+	?	1,3	
	LIF(Pleiotrophic)?	+	+	?	1,3		
	CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
15	G-CSF(Pleiotrophic)	?	+	?	?	1,3	
	IL-12(Pleiotrophic)	+	-	+	+	1,3	
20	<u>g-C family</u>						
	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
25	IL-15	?	+	?	+	5	GAS
30	<u>gp140 family</u>						
	IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5	GAS
	GM-CSF (myeloid)	-	-	+	-	5	GAS
35	<u>Growth hormone family</u>						
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
	EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
	<u>Receptor Tyrosine Kinases</u>						
	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152),

although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4<sup>+</sup> Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.



Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid**

##### **Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and

wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1 mM MgCl<sub>2</sub>, and 675 uM CaCl<sub>2</sub>. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

#### **Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor).

The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

5        The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

10       5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

15       To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

20       PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and

25       resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418

30       for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS

(Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200  $\mu$ l of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50  $\mu$ l supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ $\mu$ l of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked

5 with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

10 5':GCGGCAAGCTTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following

15 sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
TTTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTC  
CGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATG  
20 GCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTG  
AGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGC  
AAAAAGCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-  
25 promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes  
30 SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5

17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

5 Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes

in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small  
 5 molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for  
 10 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four  
 15 times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2-5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are  
 20 washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as  
 25 fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm;  
 30 and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.



### **Example 19: High-Throughput Screening Assay Identifying Tyrosine**

#### **Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar

Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

5 To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM  
10 HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane  
15 bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

20 Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a  
25 biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in  
30 order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride,

pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

- 5           The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the  
10   biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and  
15   incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

20           **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be  
25   used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by  
30   substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are

then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

### **Example 21: Method of Determining Alterations in a Gene**

#### **Corresponding to a Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre

Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

5           PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

10           Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the  
15           corresponding genomic locus.

          Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera  
20           (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and  
25           translocations. These alterations are used as a diagnostic marker for an associated disease.

#### **Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

30           A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus,

it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

### **Example 23: Formulation**

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to

modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable  
 5 polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP  
 10 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

In a preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV  
 15 compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby  
 20 incorporated by reference in their entirety. In specific preferred embodiments the Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

Examples of biodegradable polymers which can be used in the formulation of  
 25 Neutrokin-alpha and/or Neutrokin-alphaSV compositions, include but are not limited to, polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene  
 30 succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above

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materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic. These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV compositions are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV release profile (See, e.g., Ravivarapu et al., Journal of Pharmaceutical Sciences 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alcohols, diols, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alcohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and triethyl citrate. **The most preferred solvents are** N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

Additionally, formulations comprising Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV compositions and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like

hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebecate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C.sub.6 -C.sub.12 alkanols, 2-ethoxyethanol, and the like. The release rate modification agent may be used singly or in combination with other such agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to, sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

In specific preferred embodiments the Neutrokin- $\alpha$  and/or Neutrokin- $\alpha$ SV compositions of the invention are formulated using the BEMA<sup>TM</sup> BioErodible Mucoadhesive System, MCA<sup>TM</sup> MucoCutaneous Absorption System, SMP<sup>TM</sup> Solvent MicroParticle System, or BCP<sup>TM</sup> BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. (USA)* 82:3688-3692 (1985); Hwang

et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum

albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG

(e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*.

In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the

- 5 Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™
- 10 (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-
- 15 nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

- Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to
- 20 lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is
- 25 PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing
- 30 prodrug forms of  $\beta$ -L-FD4C and  $\beta$ -L-FddC (WO 98/17281).

Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A (naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a

distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , etc., may also inhibit fusion.

Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 $\alpha$ , MIP-1 $\beta$ , SDF-1 $\alpha$ , IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN- $\alpha$ 2a; antagonists of TNFs, NF $\kappa$ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines



*Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-

10  $\gamma$ -L-glutamyl-L-cysteine ethyl ester ( $\gamma$ -GCE; WO 99/56764).

ribavirin, amantadine, and remantidine.

15 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™,

20 RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™,  
CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™,  
FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™,  
KETOCONAZOLE™, ACYCLOVIR™, FAMCICLOVIR™, PYRIMETHAMINE™,  
LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™

25 (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any  
30 combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic

*Mycobacterium avium* complex infection. In another specific embodiment,

Therapeutics of the invention are used in any combination with RIFABUTIN™,

CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or

prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific

5 embodiment, Therapeutics of the invention are used in any combination with

GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat

or prevent an opportunistic cytomegalovirus infection. In another specific

embodiment, Therapeutics of the invention are used in any combination with

FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to

10 prophylactically treat or prevent an opportunistic fungal infection. In another

specific embodiment, Therapeutics of the invention are used in any combination with

ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an

opportunistic herpes simplex virus type I and/or type II infection. In another specific

embodiment, Therapeutics of the invention are used in any combination with

15 PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent

an opportunistic *Toxoplasma gondii* infection. In another specific embodiment,

Therapeutics of the invention are used in any combination with LEUCOVORIN™

and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial  
infection.

20 In a further embodiment, the Therapeutics of the invention are administered

in combination with an antibiotic agent. Antibiotic agents that may be administered

with the Therapeutics of the invention include, but are not limited to, amoxicillin,

beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases,

Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin,

25 fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin,

rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-

sulfamethoxazole, and vancomycin.

In other embodiments, Therapeutics of the invention are administered in

combination with immunosuppressive agents. Immunosuppressive agents that may

30 be administered in combination with the Therapeutics of the invention include, but are

not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide

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methyprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to, prednisolone,

5 methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid),  
 10 IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrxate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

15 In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™  
 20 (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In certain embodiments, the Therapeutics of the invention are administered  
 25 alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal,  
 30 etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin,

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phenylbutazone, piroxicam, sulindac, tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl

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complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., *Cancer Res.* 51:22-26, (1991)); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., *J. Bio. Chem.* 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., *Biochem J.* 286:475-480, (1992)); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., *Nature* 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, *J. Clin. Invest.* 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., *J. Biol. Chem.* 262(4):1659-1664, (1987)); Bisantrone (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., *Agents Actions* 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolimidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA);

Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; 5 ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

Anti-angiogenic agents that may be administed in combination with the  
 10 compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic  
 15 inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna,  
 20 St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents  
 25 that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia  
 30 Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include,

but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example,

Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouracil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES), Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one



embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are <sup>90</sup>Y and <sup>111</sup>In.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include,

but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent

Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCRIT™), stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

In certain embodiments, Therapeutics of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

In another embodiment, the Therapeutics of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amiodarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

In another embodiment, the Therapeutics of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorophenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit  $\text{Na}^+\text{-K}^+\text{-2Cl}^-$  symport (e.g., furosemide, bumetanide, azosemide, piretanide, tripamide, ethacrynic acid,

muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, canrenone, and potassium canrenoate).

In one embodiment, the Therapeutics of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to,  $^{127}\text{I}$ , radioactive isotopes of iodine such as  $^{131}\text{I}$  and  $^{123}\text{I}$ ; recombinant growth hormone, such as HUMATROPE™ (recombinant somatropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T<sub>4</sub>™, SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T<sub>3</sub>™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-*n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca<sup>2+</sup> channel blockers; dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as

ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™,

ESTRATAB™, ORTHO-EST™, OGEN™ and estropipate (estrone), ESTROVIS™

5 (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™

(estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™

(estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen),

SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™

(hydroxyprogesterone caproate), MPA™ and DEPO-PROVERA™

10 (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™

(megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and

AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT

SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™

(mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus

15 mestranol), PROGESTASERT™ (intrauterine device that releases progesterone),

LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™,

NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone),

LEVLEN™, NORDETTE™, TRI-LEVLIN™ and TRIPHASIL-21™ (ethinyl

estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel),

20 DEMULEN™ (ethinyl estradiol/ethynodiol diacetate), NORINYL™, ORTHO-

NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol),

DESOGEN™ and ORTHO-CEPT™ (ethinyl estradiol/desogestrel), ORTHO-

CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate),

MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

25 Additional treatments for endocrine and/or hormone imbalance disorders

include, but are not limited to, testosterone esters such as methenolone acetate and

testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™

(testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone

enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™

30 (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™

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and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (acclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™ (betamethasone), BENISONET™ and UTICORT™ (betamethasone benzoate), DIPROSONET™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONET™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWENT™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate),

DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASONE™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetate),

- 5 ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetate); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone).

- Additional treatments for endocrine and/or hormone imbalance disorders
- 10 include, but are not limited to bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™ (acetoheksamide), glibenclamide, MICRONASE™, DIBETA™ and
- 15 GLYNASE™ (glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), PRECOSE™ (acarbose), AMARYL™ (glimepiride), and ciglitazone; thiazolidinediones (TZDs) such as rosiglitazone, AVANDIA™ (rosiglitazone maleate) ACTOS™ (pioglitazone), and troglitazone; alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as
- 20 SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide). In still other embodiments, Therapeutics of the invention are administered in combination with one or more of the following: a biguanide antidiabetic agent, a glitazone antidiabetic agent, and a sulfonylurea antidiabetic agent.

- 25 In one embodiment, the Therapeutics of the invention are administered in combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN®
- 30 (medroxyprogesterone), MICRONOR® (norethidrone acetate), PROMETRIUM® progesterone, and megestrol acetate); and estrogen/progesterone combination

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therapies such as, for example, conjugated estrogens/medroxyprogesterone (e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estsradiol (e.g., FEMHRT™).

In an additional embodiment, the Therapeutics of the invention are

- 5 administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B<sub>12</sub>,
- 10 cyancobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

In certain embodiments, the Therapeutics of the invention are administered in combination with agents used to treat psychiatric disorders. Psychiatric drugs that

- 15 may be administered with the Therapeutics of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine,
- 20 divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g., amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranlycypromine, trazodone, trimipramine, and venlafaxine), antianxiety
- 25 agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

In other embodiments, the Therapeutics of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents

- 30 that may be administered with the Therapeutics of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate,

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gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g., levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, ropinirole, pramipexole, benztropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

In another embodiment, Therapeutics of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents. Vasodilating agents that may be administered with the Therapeutics of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril,trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the Therapeutics of the invention include, but are not limited to amlodipine, bepridil, diltiazem, felodipine, flunarizine, isradipine, nifedipine, nimodipine, and verapamil.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

#### **Example 24: Method of Treating Decreased Levels of the Polypeptide**

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

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**Example 25: Method of Treating Increased Levels of the Polypeptide**

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

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In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

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**Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

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At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

5 pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

10 The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is  
15 maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

20 The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

25 Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the  
30 media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral

vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

5 The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

**Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

10 Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935  
15 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous  
20 polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends.  
25 Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

30 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under

conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10<sup>6</sup> cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the

fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

5 Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 10 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 15 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

20 The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

#### 25 **Example 28: Method of Treatment Using Gene Therapy - In Vivo**

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide.

30 The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in

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the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., *Pharmacol. Res.* 35(6):517-522 (1997); Wolff, *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers

in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on



the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future

5 localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that

10 quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked

15 DNA.

#### **Example 29: Transgenic Animals.**

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters,

20 guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

25 Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus

30 mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic

stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993)); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989)); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific

inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

#### **Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (*e.g.*, knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (*i.e.*, animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (*e.g.*, lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, *e.g.*, by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc.

The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

### **Example 31: Production of an Antibody**

#### **Hybridoma Technology**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it

substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the

invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

*Isolation Of Antibody Fragments Directed polypeptide(s) of the invention  
From A Library Of scFvs*

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody

fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10<sup>13</sup> TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR



fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

**Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard

B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3$ H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of periaarterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

### **Example 33: T Cell Proliferation Assay**

#### **Proliferation assay for Resting PBLs.**

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 °C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells ( $5 \times 10^4$ /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 °C, plates are spun for 2 min. at 1000 rpm and 100 microliters of supernatant is removed and stored -20 °C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0.5 microcuries of  $^3\text{H}$ -thymidine and cultured at 37 °C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood lymphocytes) is measured by the up-take of  $^3\text{H}$ -thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the

plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using  $2 \times 10^4$  cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (\*), IFN  $\gamma$ , TNF  $\alpha$ , IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of  $^3\text{H}$ -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of  $^3\text{H}$ -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

(\*) The amount of the control cytokines IL-2, IFN  $\gamma$ , TNF  $\alpha$  and IL-10 produced in each transfection varies between 300pg to 5ng/ml.

#### **Costimulation assay.**

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using  $2 \times 10^4$  cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFN  $\gamma$ , TNF  $\alpha$ , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of  $^3\text{H}$ -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of  $^3\text{H}$ -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

#### **Costimulation assay: IFN $\gamma$ and IL-2 ELISA**

The assay is performed as follows. Twenty-four well plates are coated with either 300ng/ml or 600ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 500ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background

in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the costimulation assay. The assay is performed in the pre-coated twenty-four well plate using  $1 \times 10^5$  cells/well in a final volume of 900ul.

- 5 The supernatants (293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 300ul are added to 600ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only(negative control), IL-2, IFN  $\gamma$ , IL-12 and IL-18. In addition to the control supernatants recombinant human IL-2 (all cytokines were
- 10 purchased from R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml, IL-12 at a final concentration of 1ng/ml and IL-18 at a final concentration of 50ng/ml are also used. Controls and unknown samples are tested in duplicate. Supernatant samples (250ul) are collected 2 days and 5 days after the beginning of the assay. ELISAs to test for IFN  $\gamma$  and IL-2 secretion are performed using kits
- 15 purchased from R & D Systems, (Minneapolis, MN). Results are expressed as an average of duplicate samples plus or minus standard error.

#### **Proliferation assay for preactivated-resting T cells.**

- 20 A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then cultured in the presence of low doses of IL-2 resemble effector T cells. These cells
- 25 IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would have an effect on a naïve T cell. Therefore the use of this type of cells might enable to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve ) T cells.

- 30 The assay is performed as follows. PBMC are isolated by F/H gradient centrifugation from human peripheral blood, and are cultured in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the

presence of 2ug/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in 10% FCS/RPMI in the presence of 5ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are washed and rested in starvation medium (1%FCS/RPMI) for 16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using  $2 \times 10^4$  cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFN  $\gamma$ , TNF  $\alpha$ , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of  $^3\text{H}$ -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of  $^3\text{H}$ -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory

and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g, R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium



azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

5           Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be  
10 screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

15           Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA  
20 fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final  
25 concentration of 5 µg/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

30           Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml

with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRP) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H<sub>2</sub>O<sub>2</sub> produced by the macrophages, a standard curve of a H<sub>2</sub>O<sub>2</sub> solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 35: Biological Effects of Polypeptides of the Invention**

#### **Astrocyte and Neuronal Assays**

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1

and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### Fibroblast and endothelial cell assays:

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are

cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential

effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips.

- 5 The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the
- 10 factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*.

- 15 Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the

20 invention.

#### **Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

- 25 On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2.5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and
- 30 positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect**

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6

mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After

5 counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV

10 fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the

15 invention.

### **Example 39: Stimulation of Endothelial Migration**

This example will be used to explore the possibility that a polypeptide of the

20 invention may stimulate lymphatic endothelial cell migration.

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours

25 at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter

30 between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation

period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

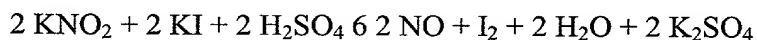
The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then



bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions.

- 5 S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

- 10 The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

15 **Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

- 20 CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 µl/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500
- 25 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 µg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in
- 30 triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer.

They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

*In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid “plug” of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson’s Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the *in vivo* effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660

(1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al. Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al. Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al. J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

#### **Example 45: Effect of Polypeptides of the Invention on Vasodilation**

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old

spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 46: Rat Ischemic Skin Flap Model**

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

Ischemic skin

Ischemic skin wounds

Normal wounds

The experimental protocol includes:

Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 47: Peripheral Arterial Disease Model**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral  
5 arterial diseases. The experimental protocol includes:

One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

10 The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the  
15 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 48: Ischemic Myocardial Disease Model**

20 A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

25 The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

30 Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 49: Rat Corneal Wound Healing Model**

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

Inserting a spatula below the lip of the incision facing the outer corner of the eye.

Making a pocket (its base is 1-1.5 mm from the edge of the eye).

Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

##### ***Diabetic db+/db+ Mouse Model.***

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal

heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study.

Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The



wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-

epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

### ***Steroid Impaired Rat Model***

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and

Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

- 5 Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$15 \quad \frac{[\text{Open area on day 8}] - [\text{Open area on day 1}]}{[\text{Open area on day 1}]}$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

25 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 51: Lymphadema Animal Model**

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving

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a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and  $\text{Ca}^{2+}$  comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

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**Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both  
10 normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium  
15 determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a  
20 stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$   
25 treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>.  
30 HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml

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penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to



test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell**

**5 Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only  
 10 a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone  
 15 has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low  
 20 level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

25 Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of  
 30 sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF

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and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l SID (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

**Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)**

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5%  $\text{CO}_2$ , 7%  $\text{O}_2$ , and 88%  $\text{N}_2$ ) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

#### **Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation**

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts

(NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50 $\mu$ g/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNF $\alpha$  is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO<sub>2</sub> until day 5.

Transfer 60 $\mu$ l from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100  $\mu$ l in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10 $\mu$ l). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100  $\mu$ l/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200  $\mu$ l/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50  $\mu$ l/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100  $\mu$ l/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100  $\mu$ l/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vascularogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the

eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

#### **Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h

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(selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

#### **Example 57: Alamar Blue Endothelial Cells Proliferation Assay**

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of



endothelial cells with slight changes in growth medium and cell concentration.

Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

5 Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM ) in triplicate wells with  
10 additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor  
15 fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth  
20 causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the  
25 output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

#### **Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction**

This assay can be used to detect and evaluate inhibition of a Mixed  
30 Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of

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adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

5 Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease,  
10 ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM<sup>®</sup>, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are  
15 adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are  
20 added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1  $\mu$ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10  $\mu$ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1  $\mu$ C of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and  
25 thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as  
30 recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous  
5 modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Additionally, the specifications and sequence listings of International Application No. PCT/US01/05614 filed February 21, 2001, and of U.S. Provisional Applications Serial Nos. 60/184,836 and 60/193,170 are all hereby incorporated by reference in their entirety.

**Table 3**

(Gene No: 30 / Clone ID: HTPBW79)

T02280" 2922650

	Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
5	Met 1	.	.	B	B	.	.	.	-0.37	0.07	*	*	.	-0.30	0.86
	Arg 2	.	.	B	B	.	.	.	0.02	0.43	*	*	.	-0.60	0.59
	Thr 3	.	.	B	B	.	.	.	-0.40	0.40	*	*	.	-0.30	0.74
	Leu 4	A	A	.	.	.	.	.	-0.82	0.66	*	*	.	-0.60	0.61
10	Phe 5	A	A	.	.	.	.	.	-0.72	0.73	*	*	.	-0.60	0.26
	Asn 6	A	A	.	.	.	.	.	-0.93	1.64	*	*	.	-0.60	0.19
	Leu 7	A	A	.	.	.	.	.	-1.63	1.84	*	*	.	-0.60	0.19
	Leu 8	A	A	.	.	.	.	.	-2.13	1.66	.	.	.	-0.60	0.22
	Trp 9	A	A	.	.	.	.	.	-1.91	1.56	.	.	.	-0.60	0.11
15	Leu 10	A	A	.	.	.	.	.	-1.88	1.66	.	.	.	-0.60	0.14
	Ala 11	A	A	.	.	.	.	.	-2.18	1.54	.	.	.	-0.60	0.09
	Leu 12	A	A	.	.	.	.	.	-1.58	1.24	.	.	.	-0.60	0.11
	Ala 13	A	A	.	.	.	.	.	-1.62	0.76	.	.	.	-0.60	0.21
	Cys 14	.	A	B	.	.	.	.	-1.37	0.71	.	.	.	-0.60	0.16
20	Ser 15	.	A	B	.	.	.	.	-0.87	0.71	.	.	.	-0.60	0.26
	Pro 16	.	.	B	B	.	.	.	-0.59	0.51	.	.	.	-0.60	0.37
	Val 17	.	.	B	B	.	.	.	-0.59	0.50	.	.	.	-0.60	1.00
	His 18	.	.	B	B	.	.	.	-0.30	0.61	*	.	F	-0.45	0.61
	Thr 19	.	.	B	B	.	.	.	0.41	0.61	*	.	F	-0.45	0.53
25	Thr 20	.	.	B	B	.	.	.	0.41	0.19	.	.	F	0.00	1.44
	Leu 21	A	.	.	B	.	.	.	0.62	-0.07	.	.	F	0.60	1.41
	Ser 22	A	.	.	.	.	T	.	0.89	-0.57	.	*	F	1.30	1.64
	Lys 23	A	.	.	.	.	T	.	0.97	-0.56	.	.	F	1.30	1.15
	Ser 24	A	.	.	.	.	T	.	1.32	-1.04	.	.	F	1.30	2.78
30	Asp 25	A	.	.	.	.	T	.	1.04	-1.73	.	.	F	1.30	4.15
	Ala 26	A	A	.	.	.	.	.	1.27	-1.61	.	.	F	0.90	2.09
	Lys 27	A	A	.	.	.	.	.	1.27	-1.11	*	.	F	0.90	1.58
	Lys 28	A	A	.	.	.	.	.	1.27	-1.11	*	*	F	0.90	1.27
	Ala 29	A	A	.	.	.	.	.	1.26	-1.11	*	.	F	0.90	2.51
35	Ala 30	A	A	.	.	.	.	.	0.44	-1.13	*	.	F	0.90	1.81
	Ser 31	A	A	.	.	.	.	.	0.22	-0.44	*	.	F	0.45	0.75
	Lys 32	A	A	.	.	.	.	.	0.18	0.24	.	.	F	-0.15	0.61
	Thr 33	A	A	.	.	.	.	.	0.18	-0.26	.	.	F	0.60	1.04
40	Leu 34	A	A	.	.	.	.	.	0.47	-0.76	.	.	F	0.90	1.56
	Leu 35	A	A	.	.	.	.	.	1.06	-0.76	.	.	F	0.90	1.04
	Glu 36	A	A	.	.	.	.	.	0.66	-0.36	.	.	F	0.60	1.25
	Lys 37	A	A	.	.	.	.	.	0.31	-0.06	.	.	F	0.94	1.32
	Ser 38	A	A	.	.	.	.	.	0.62	-0.36	.	*	F	1.28	2.14
	Gln 39	A	A	.	.	.	.	.	1.48	-1.04	.	*	F	1.92	2.06
45	Phe 40	.	.	.	.	T	T	.	2.08	-1.04	.	.	F	3.06	2.06
	Ser 41	.	.	.	.	T	T	.	1.22	-0.61	.	.	F	3.40	2.38
	Asp 42	.	.	.	.	T	T	.	1.18	-0.36	.	*	F	2.76	1.02
	Lys 43	.	.	.	.	.	T	C	1.48	-0.36	.	*	F	2.39	2.04
	Pro 44	.	.	.	.	.	.	C	1.59	-1.14	.	*	F	2.32	2.54
50	Val 45	.	.	B	.	.	.	.	1.94	-1.53	.	.	F	1.95	2.98
	Gln 46	.	.	B	.	.	.	.	1.43	-1.10	.	*	F	1.78	1.47
	Asp 47	.	.	B	.	.	T	.	0.58	-0.41	.	.	F	1.70	0.79
	Arg 48	.	.	B	.	.	T	.	-0.32	-0.20	.	.	F	1.53	0.79
	Gly 49	.	.	B	.	.	T	.	-0.42	-0.20	.	*	F	1.36	0.34
55	Leu 50	.	.	B	.	.	T	.	0.43	-0.11	.	*	.	1.04	0.29
	Val 51	.	.	B	B	.	.	.	-0.38	-0.11	.	*	.	0.47	0.25
	Val 52	.	.	B	B	.	.	.	-0.33	0.57	.	*	.	-0.60	0.21
	Thr 53	.	.	B	B	.	.	.	-1.03	0.14	.	*	F	-0.15	0.50
	Asp 54	A	.	.	B	.	.	.	-0.69	-0.04	.	*	F	0.45	0.68
60	Leu 55	A	A	.	.	.	.	.	-0.18	-0.69	.	*	F	0.90	1.59
	Lys 56	A	A	.	.	.	.	.	-0.18	-0.94	.	*	F	0.90	1.48
	Ala 57	A	A	.	.	.	.	.	-0.18	-0.79	.	*	F	0.75	0.66
	Glu 58	A	A	.	B	.	.	.	-0.68	-0.14	.	*	F	0.45	0.59

	Ser	59	A	A	.	B	.	.	-0.68	-0.14	.	*	F	0.45	0.24
	Val	60	A	A	.	B	.	.	0.10	-0.14	.	*	.	0.30	0.42
	Val	61	A	A	.	B	.	.	0.17	-0.14	.	*	.	0.30	0.33
	Leu	62	A	A	.	B	.	.	0.46	-0.14	.	*	.	0.30	0.48
5	Glu	63	A	A	.	B	.	.	0.21	-0.14	.	*	.	0.30	0.87
	His	64	A	.	.	.	.	T	-0.16	-0.03	.	*	.	0.85	1.83
	Arg	65	A	.	.	.	.	T	0.40	-0.10	.	*	.	0.85	1.19
	Ser	66	A	.	.	.	.	T	0.67	-0.40	.	*	.	0.70	0.92
	Tyr	67	A	.	.	.	.	T	1.52	0.10	.	*	.	0.10	0.68
10	Cys	68	A	A	.	.	.	.	0.93	-0.40	.	*	.	0.30	0.70
	Ser	69	A	A	.	.	.	.	1.08	0.10	.	*	.	-0.30	0.53
	Ala	70	A	A	.	.	.	.	0.97	-0.29	.	*	.	0.30	0.66
	Lys	71	A	A	.	.	.	.	1.38	-1.04	.	*	F	0.90	2.05
	Ala	72	A	A	.	.	.	.	1.59	-1.61	.	*	F	0.90	3.00
15	Arg	73	A	A	.	.	.	.	1.56	-1.50	.	*	F	0.90	4.04
	Asp	74	A	A	.	.	.	.	1.27	-1.21	.	*	F	0.90	1.75
	Arg	75	A	A	.	.	.	.	1.51	-0.71	.	*	.	0.75	1.75
	His	76	.	A	B	.	.	.	1.47	-0.79	.	*	.	0.60	0.88
	Phe	77	.	A	B	.	.	.	1.20	-0.79	.	*	.	0.60	0.88
20	Ala	78	.	A	B	.	.	.	0.28	-0.14	.	*	.	0.30	0.33
	Gly	79	.	.	B	B	.	.	-0.07	0.54	.	.	.	-0.60	0.20
	Asp	80	.	.	B	B	T	.	-0.42	0.47	*	.	.	-0.20	0.23
	Val	81	.	.	B	B	.	.	-1.24	0.44	*	*	.	-0.60	0.36
	Leu	82	.	.	B	B	.	.	-0.86	0.59	.	*	.	-0.60	0.27
25	Gly	83	.	.	B	B	.	.	-0.48	0.64	.	.	.	-0.60	0.23
	Tyr	84	.	.	B	B	.	.	-0.42	1.07	.	.	.	-0.60	0.49
	Val	85	.	.	B	B	.	.	-0.42	1.34	.	.	.	-0.60	0.62
	Thr	86	.	.	B	B	.	.	0.13	1.06	.	.	.	-0.45	1.01
	Pro	87	.	.	B	B	.	.	0.91	1.01	.	.	F	-0.45	0.86
30	Trp	88	.	.	.	.	T	.	0.91	0.76	.	.	F	0.30	1.58
	Asn	89	.	.	.	.	.	T	0.91	0.54	.	.	F	0.30	1.08
	Ser	90	.	.	.	.	.	T	1.77	0.81	*	.	.	0.15	1.10
	His	91	.	.	.	.	.	T	1.22	0.39	.	.	.	0.45	1.74
	Gly	92	.	.	.	.	T	T	1.12	0.11	*	.	.	0.50	0.80
35	Tyr	93	.	.	.	B	T	.	1.46	0.20	*	.	.	0.10	0.87
	Asp	94	.	.	B	B	.	.	0.60	-0.19	*	.	.	0.45	1.27
	Val	95	.	.	B	B	.	.	0.20	-0.04	*	.	.	0.30	0.95
	Thr	96	.	.	B	B	.	.	-0.11	0.31	*	.	.	-0.30	0.53
	Lys	97	.	.	B	B	.	.	-0.07	-0.01	*	.	F	0.45	0.31
40	Val	98	.	.	B	B	.	.	0.22	0.37	*	.	F	-0.15	0.56
	Phe	99	.	.	B	B	.	.	-0.48	-0.27	*	.	F	0.45	0.78
	Gly	100	.	.	.	B	T	.	0.07	0.03	*	.	F	0.25	0.34
	Ser	101	.	.	B	B	.	.	0.38	0.51	*	.	F	-0.45	0.66
	Lys	102	.	.	B	B	.	.	-0.56	0.27	*	.	F	0.00	1.32
45	Phe	103	.	.	B	B	T	.	0.00	0.17	.	.	F	0.25	0.93
	Thr	104	.	.	B	B	.	.	0.49	0.13	*	.	F	-0.15	0.93
	Gln	105	.	.	B	B	.	.	-0.02	0.17	.	.	F	-0.15	0.72
	Ile	106	.	.	B	B	.	.	-0.01	0.81	.	.	F	-0.45	0.62
	Ser	107	.	.	B	B	.	.	-0.87	0.94	*	.	F	-0.45	0.45
50	Pro	108	.	.	B	B	.	.	-0.17	1.14	*	.	.	-0.60	0.21
	Val	109	.	A	B	B	.	.	-0.67	1.14	.	*	.	-0.60	0.53
	Trp	110	.	A	B	B	.	.	-0.62	1.14	*	.	.	-0.60	0.33
	Leu	111	.	A	B	B	.	.	0.38	0.76	*	*	.	-0.30	0.42
55	Gln	112	.	A	B	B	.	.	0.79	0.33	*	*	.	0.45	1.11
	Leu	113	.	A	B	B	.	.	0.66	-0.31	*	*	.	1.35	2.07
	Lys	114	.	A	B	B	.	C	1.62	-0.80	*	*	F	2.30	2.49
	Arg	115	.	.	.	.	.	T	1.91	-1.49	*	*	F	3.00	2.81
	Arg	116	.	.	.	.	.	T	2.12	-1.89	*	*	F	2.70	5.90
	Gly	117	.	.	.	.	.	T	1.42	-1.96	*	*	F	2.40	2.92
60	Arg	118	.	.	.	.	.	T	2.23	-1.17	*	*	F	2.10	1.29
	Glu	119	A	A	B	.	.	.	1.33	-1.17	*	*	.	1.05	1.14
	Met	120	A	A	.	B	.	.	0.91	-0.53	*	*	.	0.60	0.86
	Phe	121	A	A	.	B	.	.	0.46	-0.47	.	*	.	0.30	0.63

5	Glu	122	A	A	.	B	.	.	-0.01	-0.04	.	*	.	0.30	0.36
	Val	123	A	A	.	B	.	.	-0.16	0.64	.	*	.	-0.60	0.30
	Thr	124	A	A	.	B	.	.	-0.16	0.53	.	.	.	-0.60	0.47
	Gly	125	A	A	.	B	.	.	-0.41	-0.26	.	*	.	0.30	0.46
	Leu	126	A	.	.	B	.	.	0.29	0.39	.	*	.	-0.30	0.46
	His	127	A	.	.	B	.	.	0.29	-0.26	.	.	.	0.30	0.53
	Asp	128	A	.	.	.	.	.	0.80	-0.34	*	.	.	0.50	0.92
	Val	129	A	.	.	.	.	.	0.82	-0.34	*	.	F	0.80	1.11
10	Asp	130	A	.	.	.	.	T	0.57	-0.11	*	.	F	0.85	0.86
	Gln	131	A	.	.	.	.	T	1.49	0.00	*	.	F	0.25	0.51
	Gly	132	A	.	.	.	.	T	0.93	0.00	*	.	F	0.40	1.34
	Trp	133	A	.	.	.	.	T	0.08	-0.14	*	.	.	0.70	0.81
	Met	134	A	A	.	.	.	.	1.04	0.50	*	.	.	-0.60	0.35
15	Arg	135	A	A	.	.	.	.	1.09	0.10	*	.	.	-0.30	0.69
	Ala	136	A	A	.	.	.	.	1.06	-0.33	*	.	.	0.45	1.30
	Val	137	A	A	.	.	.	.	0.81	-0.74	*	*	.	0.75	1.79
	Arg	138	A	A	.	.	.	.	1.14	-0.86	*	*	.	0.60	0.93
	Lys	139	A	A	.	.	.	.	1.40	-0.86	*	.	F	0.90	1.83
20	His	140	A	A	.	.	.	.	0.48	-0.93	*	.	F	0.90	2.44
	Ala	141	A	A	.	.	.	.	1.03	-0.89	*	*	F	0.90	1.03
	Lys	142	A	A	.	.	.	.	1.00	-0.39	*	*	F	0.45	0.70
	Gly	143	.	A	B	.	.	.	0.03	0.30	*	.	.	-0.30	0.36
	Leu	144	.	A	B	.	.	.	-0.22	0.44	*	.	.	-0.60	0.26
25	His	145	.	A	B	.	.	.	-0.08	0.37	*	*	.	-0.30	0.20
	Ile	146	.	A	B	.	.	.	-0.30	0.37	*	*	.	-0.30	0.41
	Val	147	.	A	B	.	.	.	-1.16	0.63	*	*	.	-0.60	0.41
	Pro	148	.	A	B	.	.	.	-1.51	0.63	*	*	.	-0.60	0.25
	Arg	149	.	A	B	.	.	.	-0.70	0.91	*	*	.	-0.60	0.30
30	Leu	150	.	A	B	.	.	.	-0.67	0.23	*	*	.	-0.30	0.71
	Leu	151	.	A	B	.	.	.	-0.07	-0.41	*	*	.	0.30	0.76
	Phe	152	.	A	B	.	.	.	0.48	0.07	*	.	.	-0.30	0.41
	Glu	153	A	A	.	.	.	.	0.44	0.56	.	*	.	-0.60	0.72
	Asp	154	.	A	.	.	.	T	0.33	0.63	.	*	.	-0.05	1.37
35	Trp	155	.	A	.	.	.	T	1.14	-0.06	.	.	.	1.19	2.63
	Thr	156	A	A	.	.	.	.	1.26	-0.84	*	*	.	1.43	2.54
	Tyr	157	.	.	.	.	.	T	2.07	-0.06	*	.	.	2.27	1.32
	Asp	158	.	.	.	.	.	T	2.07	-0.06	*	.	F	2.76	2.45
	Asp	159	.	.	.	.	.	T	1.21	-0.57	*	.	F	3.40	2.73
40	Phe	160	.	.	.	.	.	T	0.69	-0.41	*	*	F	2.76	1.29
	Arg	161	.	.	B	B	.	.	1.00	-0.49	*	*	F	1.47	0.64
	Asn	162	.	.	B	B	.	.	0.94	-0.49	*	*	.	0.98	0.64
	Val	163	.	.	.	B	.	C	0.94	-0.10	*	*	.	0.84	0.99
	Leu	164	.	.	.	B	.	C	0.94	-0.89	*	*	F	0.95	0.87
45	Asp	165	A	.	.	.	.	T	1.64	-0.89	*	*	F	1.15	0.91
	Ser	166	A	.	.	.	.	T	0.64	-1.29	*	*	F	1.30	2.12
	Glu	167	A	.	.	.	.	T	0.64	-1.24	*	.	F	1.30	1.80
	Asp	168	A	.	.	.	.	T	1.50	-1.93	*	.	F	1.30	1.87
	Glu	169	A	A	.	.	.	.	1.50	-1.93	.	*	F	0.90	2.41
50	Ile	170	A	A	.	.	.	.	1.20	-1.63	*	.	F	0.90	1.15
	Glu	171	A	A	.	.	.	.	1.54	-1.24	*	.	F	0.75	0.92
	Glu	172	A	A	.	.	.	.	1.23	-1.24	*	.	F	0.90	1.07
	Leu	173	A	A	.	.	.	.	0.38	-0.76	*	.	F	0.90	2.19
	Ser	174	A	.	.	B	.	.	-0.48	-0.80	*	.	F	0.75	0.94
55	Lys	175	A	.	.	B	.	.	0.41	-0.16	*	.	F	0.45	0.40
	Thr	176	A	.	.	B	.	.	-0.44	0.24	*	.	F	-0.15	0.85
	Val	177	A	.	.	B	.	.	-1.03	0.20	*	.	.	-0.30	0.47
	Val	178	A	.	.	B	.	.	-0.18	0.31	*	.	.	-0.30	0.24
	Gln	179	A	.	.	B	.	.	0.12	0.31	*	.	.	-0.30	0.33
60	Val	180	A	.	.	B	.	.	0.08	0.23	*	.	.	-0.30	0.71
	Ala	181	.	.	B	B	.	.	0.36	-0.01	.	.	.	0.45	1.66
	Lys	182	A	.	.	B	.	.	0.51	-0.16	.	.	F	0.70	1.30
	Asn	183	.	.	B	.	.	.	1.37	0.23	.	.	F	0.40	1.52
	Gln	184	.	.	B	.	.	.	1.02	-0.41	.	.	F	1.10	2.51

	His	185	.	.	.	.	.	.	T	T	C	1.18	-0.49	.	.	F	1.60	1.24
	Phe	186	.	.	.	.	.	.	T	T	.	0.91	0.30	.	.	.	1.00	0.67
	Asp	187	.	.	B	.	.	.	T	T	.	0.01	0.54	.	.	.	0.20	0.29
5	Gly	188	.	.	B	.	.	.	T	T	.	0.01	0.79	*	.	.	0.10	0.16
	Phe	189	.	.	B	B	.	.	.	.	.	-0.84	0.29	*	.	.	-0.10	0.31
	Val	190	.	.	B	B	.	.	.	.	.	-1.10	0.14	*	.	.	-0.20	0.14
	Val	191	.	.	B	B	.	.	.	.	.	-0.40	1.06	*	.	.	-0.60	0.15
	Glu	192	A	.	.	B	.	.	.	.	.	-0.40	1.03	*	.	.	-0.60	0.27
	Val	193	A	.	.	B	.	.	.	.	.	-0.87	0.64	*	.	.	-0.60	0.64
10	Trp	194	A	.	.	B	.	.	.	.	.	-0.98	0.69	*	.	.	-0.60	0.71
	Asn	195	A	.	.	B	.	.	.	.	.	-0.42	0.73	*	.	.	-0.60	0.34
	Gln	196	A	.	.	B	.	.	.	.	.	0.43	1.11	.	.	.	-0.60	0.61
	Leu	197	A	.	.	B	.	.	.	.	.	0.48	0.87	*	.	F	-0.30	1.01
	Leu	198	A	.	.	B	.	.	.	.	.	1.44	-0.04	.	.	F	0.78	1.25
15	Ser	199	.	.	.	B	.	.	.	C	.	0.88	-0.44	.	*	F	1.16	1.42
	Gln	200	.	.	.	B	B	T	.	.	.	0.57	-0.20	.	.	F	1.54	1.28
	Lys	201	.	.	B	B	.	.	.	.	.	0.57	-0.40	.	*	F	1.32	2.23
	Arg	202	.	.	B	B	.	.	.	.	.	1.38	-1.09	*	*	F	1.80	2.78
	Val	203	.	.	B	B	.	.	.	.	.	1.38	-1.07	*	.	F	1.62	2.78
20	Thr	204	.	.	B	B	.	.	.	.	.	1.33	-0.79	*	*	F	1.44	1.15
	Asp	205	.	.	B	.	.	.	T	.	.	0.73	-0.36	*	*	F	1.21	0.58
	Gln	206	A	.	.	.	.	.	T	.	.	-0.01	0.26	*	.	.	0.28	0.77
	Leu	207	A	.	.	.	.	.	T	.	.	-0.43	0.40	*	*	.	-0.20	0.46
25	Gly	208	A	.	.	.	.	.	T	.	.	0.39	0.40	*	.	.	-0.20	0.40
	Met	209	A	A	.	.	.	.	.	.	.	0.74	0.90	.	.	.	-0.60	0.31
	Phe	210	A	A	.	.	.	.	.	.	.	0.74	0.50	.	.	.	-0.60	0.76
	Thr	211	A	A	.	.	.	.	.	.	.	0.04	-0.19	*	.	.	0.45	1.34
	His	212	A	A	.	.	.	.	.	.	.	0.86	0.17	.	.	.	-0.15	1.17
30	Lys	213	A	A	.	.	.	.	.	.	.	1.20	-0.44	*	.	F	0.60	2.34
	Glu	214	A	A	.	.	.	.	.	.	.	0.99	-0.83	*	.	F	0.90	2.81
	Phe	215	A	A	.	.	.	.	.	.	.	1.10	-0.63	*	.	F	0.90	1.70
	Glu	216	A	A	.	.	.	.	.	.	.	1.20	-0.63	*	.	F	0.75	0.86
	Gln	217	A	A	.	.	.	.	.	.	.	0.38	-0.20	*	.	.	0.30	0.77
35	Leu	218	A	A	.	.	.	.	.	.	.	-0.48	0.44	*	.	.	-0.60	0.66
	Ala	219	A	A	.	.	.	.	.	.	.	-0.48	0.34	*	.	.	-0.30	0.31
	Pro	220	A	.	.	.	.	.	.	.	.	-0.12	0.34	*	.	.	-0.10	0.30
	Val	221	A	.	.	.	.	.	.	.	.	-0.82	0.37	*	.	.	-0.10	0.36
	Leu	222	A	.	.	.	.	.	.	.	.	-1.12	0.47	.	.	.	-0.40	0.31
40	Asp	223	A	.	.	.	.	.	T	.	.	-1.12	0.36	.	.	.	0.10	0.27
	Gly	224	.	.	B	.	.	.	T	.	.	-1.13	0.61	.	.	.	-0.20	0.30
	Phe	225	.	.	B	.	.	.	T	.	.	-1.23	0.59	.	.	.	-0.20	0.36
	Ser	226	.	.	B	.	.	.	T	.	.	-0.62	0.39	.	.	.	0.10	0.31
	Leu	227	.	.	B	.	.	.	.	.	.	0.19	1.14	.	.	.	-0.40	0.49
45	Met	228	.	.	B	.	.	.	.	.	.	-0.06	0.71	.	.	.	-0.40	0.95
	Thr	229	.	.	B	.	.	.	T	.	.	-0.01	0.69	.	.	.	-0.05	1.11
	Tyr	230	.	.	.	.	.	T	T	.	.	0.38	0.69	.	.	.	0.35	1.80
	Asp	231	.	.	.	.	.	T	T	.	.	0.09	0.49	.	.	.	0.35	2.62
	Tyr	232	.	.	.	.	.	T	T	.	.	0.87	0.37	.	.	.	0.65	1.84
	Ser	233	.	.	B	.	.	.	.	.	.	1.47	0.39	.	.	.	0.05	1.59
50	Thr	234	.	.	B	.	.	.	.	.	.	1.57	0.03	.	.	.	0.05	1.65
	Ala	235	.	.	B	.	.	.	.	.	.	1.47	0.46	.	.	.	-0.25	1.63
	His	236	.	.	B	.	.	.	.	.	.	1.26	0.13	.	.	F	0.20	1.21
	Gln	237	.	.	.	.	.	.	.	.	C	1.50	0.17	.	.	F	0.40	1.29
	Pro	238	.	.	.	.	.	.	.	.	C	1.21	0.09	.	.	F	0.40	2.06
55	Gly	239	.	.	.	.	.	.	T	.	C	1.31	0.09	.	.	F	0.60	1.53
	Pro	240	.	.	.	.	.	T	T	.	.	1.09	0.01	.	.	F	0.80	1.36
	Asn	241	.	.	.	.	.	.	T	.	C	0.82	0.30	.	*	F	0.45	0.73
	Ala	242	.	.	.	.	.	.	T	.	C	0.53	0.26	.	.	F	0.45	0.98
60	Pro	243	.	.	B	.	.	.	.	.	.	-0.11	0.74	*	.	.	-0.40	0.67
	Leu	244	.	.	B	B	.	.	.	.	.	0.34	0.96	*	.	.	-0.60	0.31
	Ser	245	.	.	B	B	.	.	.	.	.	-0.03	0.56	*	.	.	-0.60	0.60
	Trp	246	.	.	B	B	.	.	.	.	.	-0.70	0.56	*	*	.	-0.60	0.39
	Val	247	.	.	B	B	.	.	.	.	.	-0.97	0.70	*	*	.	-0.60	0.25

5	Arg	248	.	.	B	B	.	.	.	-0.76	0.66	*	*	.	-0.60	0.14
	Ala	249	.	.	B	B	.	.	.	-0.80	0.67	*	*	.	-0.60	0.23
	Cys	250	.	.	B	B	.	.	.	-1.31	0.40	*	*	.	-0.60	0.23
	Val	251	.	.	B	B	.	.	.	-1.02	0.44	*	*	.	-0.60	0.10
	Gln	252	.	.	B	B	.	.	.	-0.38	0.44	*	*	.	-0.60	0.16
10	Val	253	.	.	B	B	.	.	.	-0.44	0.37	*	*	.	0.04	0.47
	Leu	254	.	.	B	B	.	.	.	-0.16	-0.20	.	*	.	1.13	1.25
	Asp	255	.	.	B	.	.	T	.	0.56	-0.46	.	*	F	1.87	0.97
	Pro	256	.	.	.	.	T	T	.	1.12	-0.86	.	*	F	3.06	2.62
	Lys	257	.	.	.	.	T	T	.	1.23	-0.59	.	*	F	3.40	3.34
15	Ser	258	A	.	.	.	.	T	.	1.79	-1.27	.	*	F	2.66	3.91
	Lys	259	A	.	.	.	.	.	.	2.64	-0.89	.	*	F	2.12	3.39
	Trp	260	A	.	.	.	.	T	.	1.76	-1.31	.	*	F	1.98	3.39
	Arg	261	A	.	.	.	.	T	.	1.16	-0.63	.	*	F	1.64	1.77
	Ser	262	.	.	B	.	.	T	.	0.30	-0.33	.	*	F	0.85	0.73
20	Lys	263	.	.	B	.	.	T	.	0.26	0.36	.	*	F	0.25	0.57
	Ile	264	.	.	B	B	.	.	.	-0.60	-0.13	.	*	.	0.30	0.29
	Leu	265	.	.	B	B	.	.	.	-0.31	0.56	.	*	.	-0.60	0.18
	Leu	266	.	.	B	B	.	.	.	-1.12	0.57	.	*	.	-0.60	0.14
	Gly	267	.	.	B	B	.	.	.	-1.07	1.36	.	*	.	-0.60	0.18
25	Leu	268	.	.	B	.	.	.	.	-1.46	1.43	.	*	.	-0.40	0.34
	Asn	269	.	.	B	.	.	.	.	-1.17	1.17	.	*	.	-0.40	0.40
	Phe	270	.	.	B	.	.	.	.	-0.36	1.10	.	.	.	-0.40	0.40
	Tyr	271	.	.	B	.	.	.	.	0.21	0.67	.	*	.	-0.40	0.82
	Gly	272	.	.	B	.	.	T	.	-0.03	0.74	.	.	.	-0.20	0.80
30	Met	273	.	.	B	.	.	T	.	0.47	0.84	.	.	.	-0.20	0.93
	Asp	274	.	.	B	.	.	T	.	0.17	0.54	.	.	.	-0.20	0.86
	Tyr	275	A	.	.	.	.	T	.	0.91	0.17	.	.	.	0.25	1.16
	Ala	276	A	.	.	.	.	.	.	1.16	-0.26	.	.	.	0.65	2.34
	Thr	277	A	.	.	.	.	.	.	0.91	-0.87	.	*	F	1.10	2.34
35	Ser	278	A	.	.	.	.	T	.	1.62	-0.37	*	.	F	1.00	1.51
	Lys	279	A	.	.	.	.	T	.	1.62	-1.13	.	.	F	1.30	2.93
	Asp	280	A	.	.	.	.	T	.	1.66	-1.63	*	*	F	1.30	3.52
	Ala	281	A	.	.	.	.	T	.	1.39	-1.69	.	.	F	1.30	4.06
	Arg	282	.	.	B	.	.	.	.	0.84	-1.43	.	.	F	1.10	1.51
40	Glu	283	.	.	B	B	.	.	.	0.80	-0.79	*	.	F	0.75	0.67
	Pro	284	.	.	B	B	.	.	.	0.17	-0.36	*	.	F	0.45	0.66
	Val	285	.	.	B	B	.	.	.	0.28	-0.36	*	*	.	0.30	0.34
	Val	286	.	.	B	B	.	.	.	0.62	-0.36	*	.	.	0.30	0.38
	Gly	287	.	.	B	.	.	T	.	-0.38	0.40	*	.	.	-0.20	0.39
45	Ala	288	.	.	B	.	.	T	.	-0.38	0.66	.	.	.	-0.20	0.37
	Arg	289	.	.	B	.	.	T	.	-0.48	0.41	*	.	.	-0.20	0.85
	Tyr	290	.	.	B	.	.	T	.	-0.43	0.26	*	*	.	0.25	1.25
	Ile	291	.	A	B	B	.	.	.	0.47	0.51	*	*	.	-0.45	1.02
	Gln	292	.	A	B	B	.	.	.	0.81	0.01	*	.	.	-0.15	1.04
50	Thr	293	.	A	B	B	.	.	.	1.37	0.01	*	*	F	0.00	1.11
	Leu	294	.	A	B	B	.	.	.	1.37	-0.24	*	.	F	0.90	2.15
	Lys	295	.	A	.	B	.	.	.	1.40	-0.93	.	*	F	1.90	2.43
	Asp	296	.	A	.	.	T	.	.	2.40	-0.90	.	*	F	2.20	2.60
	His	297	.	A	.	.	.	.	C	1.80	-1.39	.	*	F	2.30	6.18
55	Arg	298	.	.	.	.	.	T	C	1.26	-1.46	.	*	F	3.00	3.06
	Pro	299	.	.	B	.	.	T	.	1.78	-0.81	.	*	F	2.50	1.36
	Arg	300	.	.	B	.	.	T	.	1.73	0.10	.	*	.	1.15	1.05
	Met	301	.	.	B	.	.	T	.	1.43	-0.40	.	*	.	1.30	0.90
	Val	302	.	.	B	.	.	.	.	1.47	-0.01	.	*	.	0.80	0.78
60	Trp	303	.	.	B	.	.	T	.	0.97	-0.04	.	*	.	0.70	0.69
	Asp	304	.	.	.	.	.	T	C	0.88	0.39	.	*	F	0.45	0.89
	Ser	305	.	.	.	.	.	T	C	0.77	0.16	.	*	F	0.60	1.60
	Gln	306	.	.	.	.	.	T	C	1.33	-0.49	.	.	F	1.20	2.64
	Xxx	307	.	A	.	.	.	.	C	1.49	-0.90	.	.	F	1.10	2.15
60	Ser	308	A	A	.	.	.	.	.	1.08	-0.11	.	.	F	0.60	1.39
	Glu	309	A	A	.	.	.	.	.	1.08	0.29	.	.	F	-0.15	0.69
	His	310	A	A	.	.	.	.	.	1.13	-0.11	.	.	.	0.30	0.94



	Phe	311	A	A	.	.	.	.	.	1.18	0.21	.	.	.	-0.15	1.10
	Phe	312	A	A	.	.	.	.	.	1.61	-0.17	.	.	.	0.45	1.27
	Glu	313	A	A	.	.	.	.	.	1.61	-0.17	.	.	.	0.79	1.87
	Tyr	314	A	A	.	.	.	.	.	1.72	-0.29	.	.	.	1.13	2.89
5	Lys	315	A	A	.	.	.	.	.	1.46	-1.07	.	.	.	1.92	6.53
	Lys	316	.	A	.	.	.	T	.	1.81	-1.47	.	.	F	2.66	5.05
	Ser	317	.	.	.	.	.	T	T	2.62	-1.04	*	.	F	3.40	3.19
	Arg	318	.	.	.	.	.	T	T	2.59	-1.80	*	.	F	3.06	3.13
	Ser	319	.	.	.	.	.	T	T	1.98	-1.30	*	.	F	2.72	2.13
10	Gly	320	.	.	.	.	.	T	T	1.08	-0.66	*	.	F	2.38	1.18
	Arg	321	.	.	B	B	.	.	.	0.33	-0.40	.	.	F	0.79	0.45
	His	322	.	.	B	B	.	.	.	0.39	0.39	*	.	.	-0.30	0.29
	Val	323	.	.	B	B	.	.	.	0.07	0.76	*	.	.	-0.60	0.46
	Val	324	.	.	B	B	.	.	.	0.06	0.76	.	.	.	-0.60	0.36
15	Phe	325	.	.	B	B	.	.	.	-0.41	1.24	.	*	.	-0.60	0.38
	Tyr	326	.	.	B	B	.	.	.	-0.48	1.43	.	*	.	-0.60	0.42
	Pro	327	.	.	B	B	.	.	.	-0.74	0.79	.	.	F	-0.30	1.14
	Thr	328	.	A	.	.	.	T	.	-0.70	0.53	.	.	F	0.10	1.77
	Leu	329	A	A	.	.	.	.	.	0.16	0.43	*	.	F	-0.45	0.93
20	Lys	330	A	A	.	.	.	.	.	0.00	0.07	.	*	F	0.00	1.04
	Ser	331	A	A	.	.	.	.	.	0.36	0.29	.	*	F	-0.15	0.54
	Leu	332	A	A	.	.	.	.	.	-0.24	-0.20	.	*	.	0.45	1.28
	Gln	333	.	A	B	.	.	.	.	0.07	-0.20	.	*	.	0.30	0.53
25	Val	334	.	A	B	.	.	.	.	0.07	-0.20	.	*	.	0.30	0.68
	Arg	335	A	A	.	.	.	.	.	-0.57	0.10	.	*	.	-0.30	0.68
	Leu	336	A	A	.	.	.	.	.	-0.16	-0.09	.	*	.	0.30	0.40
	Glu	337	A	A	.	.	.	.	.	0.66	-0.49	*	*	.	0.45	1.05
	Leu	338	A	A	.	.	.	.	.	-0.16	-1.13	.	*	.	0.60	0.93
	Ala	339	A	A	.	.	.	.	.	0.36	-0.44	.	*	.	0.30	0.93
30	Arg	340	A	A	.	.	.	.	.	-0.61	-0.70	.	*	.	0.60	0.53
	Glu	341	A	A	.	B	.	.	.	-0.14	-0.06	*	.	.	0.30	0.48
	Leu	342	A	A	.	B	.	.	.	-1.00	-0.31	*	.	.	0.30	0.47
	Gly	343	A	A	.	B	.	.	.	-0.49	-0.17	*	*	.	0.30	0.18
	Val	344	.	.	B	B	.	.	.	-0.79	0.21	*	.	.	-0.30	0.14
35	Gly	345	.	.	B	B	.	.	.	-1.19	0.90	*	.	.	-0.60	0.12
	Val	346	.	.	B	B	.	.	.	-1.19	1.13	.	*	.	-0.60	0.12
	Ser	347	.	.	B	B	.	.	.	-1.19	0.70	.	.	.	-0.60	0.29
	Ile	348	.	.	B	B	.	.	.	-1.19	0.74	.	.	.	-0.60	0.24
	Trp	349	.	.	B	B	.	.	.	-0.33	0.74	.	.	.	-0.60	0.32
40	Glu	350	.	.	B	B	.	.	.	-0.33	0.50	*	.	.	-0.51	0.41
	Leu	351	.	.	B	.	.	.	.	-0.29	0.54	.	.	.	-0.22	0.58
	Gly	352	.	.	.	.	.	T	T	0.01	0.54	*	.	F	0.62	0.46
	Gln	353	.	.	.	.	.	T	T	0.66	-0.37	*	.	F	1.61	0.44
45	Gly	354	.	.	.	.	.	.	C	0.24	0.39	*	.	F	0.90	0.84
	Leu	355	.	.	.	.	.	T	C	0.00	0.49	*	.	.	0.36	0.73
	Asp	356	.	.	B	B	.	.	.	0.81	0.81	*	.	.	-0.33	0.66
	Tyr	357	.	.	B	B	.	.	.	0.34	0.41	*	.	.	-0.27	1.12
	Phe	358	.	A	B	B	.	.	.	-0.47	0.67	*	.	.	-0.36	1.12
	Tyr	359	.	A	B	B	.	.	.	-0.51	0.67	*	.	.	-0.60	0.55
50	Asp	360	.	A	B	B	.	.	.	-0.09	1.10	*	.	.	-0.60	0.45
	Leu	361	.	A	B	B	.	.	.	-0.48	0.77	*	.	.	-0.60	0.67
	Leu	362	A	A	.	B	.	.	.	-0.62	0.41	.	.	.	-0.60	0.54

**Table 4**

(Gene No: 113 / Clone ID: HCE3Q10)

5	Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
	Met 1	.	.	B	.	.	.	.	-0.39	0.26	.	.	.	-0.10	0.57
	Gly 2	A	.	.	.	.	.	.	-0.59	0.33	.	.	.	-0.10	0.45
	Ala 3	A	A	.	.	.	.	.	-0.50	0.40	.	.	.	-0.30	0.36
10	Pro 4	A	A	.	.	.	.	.	-0.92	0.36	.	.	.	-0.30	0.48
	Ala 5	A	A	.	.	.	.	.	-1.34	0.43	.	.	.	-0.60	0.40
	Ala 6	A	A	.	.	.	.	.	-1.56	0.69	.	.	.	-0.60	0.33
	Ser 7	A	A	.	.	.	.	.	-2.02	0.87	.	.	.	-0.60	0.18
	Leu 8	A	A	.	.	.	.	.	-2.24	1.13	.	.	.	-0.60	0.14
15	Leu 9	A	A	.	.	.	.	.	-2.84	1.31	.	.	.	-0.60	0.12
	Leu 10	A	A	.	.	.	.	.	-3.07	1.50	.	.	.	-0.60	0.07
	Leu 11	A	A	.	.	.	.	.	-3.18	1.80	.	.	.	-0.60	0.07
	Leu 12	A	A	.	.	.	.	.	-3.47	1.90	.	.	.	-0.60	0.08
	Leu 13	A	A	.	.	.	.	.	-3.32	1.71	.	.	.	-0.60	0.09
20	Leu 14	A	A	.	.	.	.	.	-3.18	1.60	.	.	.	-0.60	0.06
	Phe 15	.	A	B	.	.	.	.	-2.66	1.49	.	.	.	-0.60	0.04
	Ala 16	.	A	B	.	.	.	.	-2.43	1.71	.	.	.	-0.60	0.05
	Cys 17	.	A	B	.	.	.	.	-1.83	1.53	.	.	.	-0.60	0.06
	Cys 18	.	A	B	.	.	.	.	-1.37	1.27	.	.	.	-0.60	0.11
25	Trp 19	.	A	B	.	.	.	.	-0.90	0.91	.	.	.	-0.60	0.11
	Ala 20	.	.	.	.	.	T	C	-0.79	0.84	.	.	.	0.00	0.20
	Pro 21	.	.	.	.	T	T	.	-0.20	0.77	*	.	F	0.35	0.37
	Gly 22	.	.	.	.	T	T	.	-0.34	0.60	.	.	F	0.35	0.57
	Gly 23	.	.	.	.	T	T	.	0.02	0.37	*	.	F	0.65	0.47
30	Ala 24	.	.	.	.	.	.	C	0.31	0.26	*	.	F	0.25	0.40
	Asn 25	.	.	.	.	.	.	C	0.90	0.23	*	*	F	0.25	0.71
	Leu 26	.	.	B	.	.	.	.	0.77	-0.20	*	*	F	0.80	1.19
	Ser 27	.	.	B	.	.	T	.	0.87	-0.20	.	*	F	1.00	1.17
	Gln 28	.	.	.	.	T	T	.	0.92	0.06	.	*	F	0.80	1.14
35	Asp 29	.	.	.	.	T	T	.	1.51	0.57	.	*	F	0.50	1.45
	Gly 30	.	.	.	.	.	T	C	1.51	0.29	.	*	F	0.60	1.87
	Tyr 31	.	A	.	.	T	.	.	2.32	-0.10	.	.	.	0.85	1.87
	Trp 32	.	A	B	.	.	.	.	2.62	-0.10	.	.	F	0.60	1.94
	Gln 33	.	A	B	.	.	.	.	1.81	-0.10	.	.	F	0.60	3.28
40	Glu 34	.	A	B	.	.	.	.	1.81	0.16	.	*	F	0.00	1.72
	Gln 35	A	A	.	.	.	.	.	1.34	-0.60	.	.	F	0.90	2.84
	Asp 36	A	A	.	.	.	.	.	1.24	-0.83	.	.	F	0.90	1.35
	Leu 37	A	A	.	.	.	.	.	1.22	-0.80	.	.	F	0.75	0.77
	Glu 38	A	A	.	.	.	.	.	0.41	-0.31	.	.	F	0.45	0.64
45	Leu 39	A	A	.	.	.	.	.	-0.18	-0.03	.	*	.	0.30	0.32
	Gly 40	A	A	.	.	.	.	.	-0.39	0.47	.	.	.	-0.60	0.39
	Thr 41	A	A	.	.	.	.	.	-1.20	0.21	.	*	.	-0.30	0.35
	Leu 42	A	A	.	.	.	.	.	-0.39	0.90	.	.	.	-0.60	0.35
	Ala 43	A	A	.	.	.	.	.	-0.39	0.21	.	.	.	-0.30	0.59
50	Pro 44	A	A	.	.	.	.	.	-0.17	-0.21	*	.	.	0.30	0.70
	Leu 45	A	A	.	.	.	.	.	-0.71	-0.20	*	.	.	0.30	0.86
	Asp 46	A	A	.	.	.	.	.	-0.70	-0.20	*	.	.	0.30	0.60
	Glu 47	A	A	.	.	.	.	.	-0.19	-0.31	*	.	.	0.30	0.52
	Ala 48	A	.	.	B	.	.	.	0.09	-0.36	*	*	.	0.30	0.84
55	Ile 49	.	.	B	B	.	.	.	-0.56	-0.56	*	.	F	0.75	0.73
	Ser 50	.	.	B	B	.	.	.	-0.03	0.09	*	.	F	-0.15	0.31
	Ser 51	.	.	B	B	.	.	.	-0.33	1.00	.	.	F	-0.45	0.33
	Thr 52	.	.	B	B	.	.	.	-0.63	0.89	.	.	F	-0.45	0.62
	Val 53	.	.	.	B	T	.	.	-0.26	0.59	*	.	F	-0.05	0.62
60	Trp 54	.	.	.	B	T	.	.	0.63	0.63	*	.	F	-0.05	0.72
	Ser 55	.	.	.	B	.	.	C	0.33	0.24	*	.	F	0.05	0.83
	Ser 56	.	.	.	.	.	T	C	-0.18	0.37	*	.	F	0.60	1.11

T02290" 29/EE66

T02280" 49222660

	Pro	57	.	.	.	.	.	.	T	T	C	-0.46	0.41	*	.	F	0.15	0.87
	Asp	58	.	.	.	.	.	T	T	.	.	0.10	0.00	*	.	F	1.25	0.65
	Met	59	.	.	B	.	.	.	T	.	.	0.39	0.00	.	.	.	0.70	0.65
5	Leu	60	.	.	B	.	.	.	.	.	.	0.69	0.01	.	.	.	0.24	0.73
	Ala	61	.	.	B	.	.	.	.	.	.	0.69	-0.41	.	.	.	1.18	0.73
	Ser	62	.	.	B	.	.	.	T	.	.	0.90	-0.03	.	.	F	1.87	0.99
	Gln	63	.	.	.	.	.	T	T	.	.	0.69	-0.24	.	.	F	2.76	2.08
	Asp	64	.	.	.	.	.	T	T	.	.	1.00	-0.50	.	.	F	3.40	3.19
10	Ser	65	.	.	.	.	.	.	T	C	.	1.50	-0.09	*	.	F	2.56	2.50
	Gln	66	.	.	.	.	.	.	.	C	.	1.79	0.01	.	.	F	1.66	2.08
	Pro	67	.	.	.	.	.	T	.	.	.	2.09	0.00	.	.	F	2.36	1.67
	Trp	68	.	.	.	.	.	.	.	C	.	2.09	0.00	.	.	F	2.06	2.08
	Thr	69	.	.	.	.	.	.	T	C	.	1.78	-0.39	.	.	F	2.16	2.08
15	Ser	70	.	.	.	.	.	.	T	C	.	1.22	-0.30	.	.	F	2.40	1.95
	Asp	71	.	.	B	.	.	.	T	.	.	0.37	-0.09	.	.	F	1.96	1.37
	Glu	72	.	.	B	.	.	.	T	.	.	-0.01	-0.36	.	.	F	1.57	0.71
	Thr	73	.	.	B	.	.	.	.	.	.	-0.07	-0.34	.	.	F	1.13	0.53
	Val	74	.	.	B	.	.	.	.	.	.	-0.10	-0.30	.	.	.	0.74	0.32
20	Val	75	.	.	B	.	.	.	T	.	.	-0.11	0.13	.	.	.	0.10	0.18
	Ala	76	A	.	.	.	.	.	T	.	.	-0.97	0.61	.	.	.	-0.20	0.18
	Gly	77	A	.	.	.	.	.	T	.	.	-1.82	0.77	.	.	F	-0.05	0.18
	Gly	78	A	.	.	.	.	.	T	.	.	-2.32	0.77	*	*	F	-0.05	0.18
25	Thr	79	A	.	.	.	.	.	.	.	.	-1.42	0.81	*	*	F	-0.45	0.15
	Val	80	A	.	.	B	.	.	.	.	.	-1.23	0.31	.	*	.	-0.30	0.30
	Val	81	.	.	B	B	.	.	.	.	.	-0.64	0.46	.	*	.	-0.60	0.16
	Leu	82	.	.	B	B	B	.	.	.	.	-1.16	0.43	*	*	.	-0.60	0.19
	Lys	83	.	.	B	B	B	.	.	.	.	-0.77	0.59	*	*	.	-0.60	0.19
30	Cys	84	.	.	B	B	B	.	.	.	.	-0.46	-0.06	.	*	.	0.30	0.52
	Gln	85	A	.	.	B	B	.	.	.	.	0.37	-0.70	.	*	.	0.75	1.05
	Val	86	A	.	.	B	B	.	.	.	.	1.22	-0.89	*	*	.	0.60	0.72
	Lys	87	.	.	B	B	.	.	.	.	.	2.03	-0.89	*	*	F	1.24	2.32
35	Asp	88	A	.	.	.	.	.	.	.	.	1.69	-1.46	.	*	F	1.78	2.24
	His	89	A	.	.	.	.	.	.	.	.	2.06	-1.47	.	*	F	2.12	4.04
	Glu	90	A	.	.	.	.	.	.	.	.	1.24	-1.73	*	*	F	2.46	2.71
	Asp	91	.	.	.	.	.	T	T	.	.	2.10	-1.04	*	*	F	3.40	1.34
40	Ser	92	.	.	.	.	.	T	T	.	.	1.77	-0.64	.	*	F	3.06	1.70
	Ser	93	.	.	.	.	.	T	T	.	.	1.47	-0.23	.	.	F	2.42	1.03
	Leu	94	.	.	.	.	.	T	T	.	.	1.50	0.16	.	*	.	1.18	0.83
	Gln	95	.	.	.	.	.	T	T	.	.	1.29	0.56	*	*	.	0.34	0.99
	Trp	96	.	.	.	.	.	T	.	.	.	0.70	0.60	*	*	.	0.15	1.15
45	Ser	97	.	.	.	.	.	.	.	C	.	1.00	0.71	*	*	F	0.10	1.41
	Asn	98	.	.	.	.	.	.	T	C	.	1.30	0.43	*	*	F	0.30	1.41
	Pro	99	.	.	.	.	.	.	T	C	.	1.80	0.43	*	*	F	0.30	2.31
	Ala	100	.	.	.	.	.	T	T	.	.	0.99	0.00	*	.	F	1.40	2.49
50	Gln	101	.	.	B	.	.	.	T	.	.	1.03	0.30	*	.	F	0.40	1.28
	Gln	102	.	.	B	B	.	.	.	.	.	0.63	0.66	*	.	F	-0.30	1.30
	Thr	103	.	.	B	B	B	.	.	.	.	0.29	1.01	*	.	F	-0.30	1.11
	Leu	104	.	.	B	B	B	.	.	.	.	0.50	0.94	*	.	.	-0.60	0.63
55	Tyr	105	.	.	B	.	.	.	.	.	.	1.13	0.54	*	.	.	-0.40	0.63
	Phe	106	.	A	B	.	.	.	.	.	.	1.24	0.14	*	.	.	-0.30	0.88
	Gly	107	A	A	.	.	.	.	.	.	.	0.66	-0.34	*	.	F	0.60	2.09
	Glu	108	A	A	.	.	.	.	.	.	.	0.16	-0.53	*	.	F	0.90	1.35
	Lys	109	A	A	.	.	.	.	.	.	.	1.08	-0.60	*	.	F	0.90	1.28
60	Arg	110	A	A	.	.	.	.	.	.	.	1.32	-1.39	*	.	F	0.90	2.54
	Ala	111	A	A	.	.	.	.	.	.	.	2.02	-1.81	*	.	F	0.90	2.45
	Leu	112	A	A	.	.	.	.	.	.	.	2.48	-1.41	*	.	F	0.90	1.97
	Arg	113	A	.	.	.	.	.	T	.	.	1.59	-1.41	*	*	F	1.30	1.97
	Asp	114	A	.	.	.	.	.	T	.	.	1.54	-0.73	*	*	F	1.30	1.37
	Asn	115	A	.	.	.	.	.	T	.	.	0.62	-0.83	*	*	F	1.30	2.87
	Arg	116	.	.	B	.	.	.	T	.	.	0.36	-0.83	.	*	F	1.30	1.21
	Ile	117	.	.	B	B	.	.	.	.	.	0.86	-0.19	.	*	.	0.30	0.54
	Gln	118	.	.	B	B	.	.	.	.	.	0.44	0.30	.	*	.	-0.30	0.48
	Leu	119	.	.	B	B	.	.	.	.	.	0.13	0.29	.	*	.	-0.30	0.33

	Val	120	.	.	B	B	.	.	-0.08	0.77	.	*	.	-0.36	0.68
	Thr	121	.	.	B	B	.	.	-0.22	0.51	.	*	F	0.03	0.61
	Ser	122	.	.	B	.	.	.	0.67	0.61	*	.	F	0.47	1.00
5	Thr	123	.	.	.	.	.	T	-0.14	-0.07	*	.	F	2.16	2.33
	Pro	124	.	.	.	.	.	T	0.37	-0.03	.	*	F	2.40	1.33
	His	125	.	.	.	.	.	T	0.33	-0.13	.	*	F	2.16	1.33
	Glu	126	.	.	B	.	.	T	0.34	0.17	.	*	.	0.82	0.65
	Leu	127	.	.	B	B	.	.	-0.24	0.07	.	*	.	0.18	0.56
10	Ser	128	.	.	B	B	.	.	-0.23	0.33	*	*	.	-0.06	0.29
	Ile	129	.	.	B	B	.	.	-0.02	0.21	*	*	.	-0.30	0.22
	Ser	130	.	.	B	B	.	.	-0.84	0.61	*	*	.	-0.60	0.44
	Ile	131	.	.	B	B	.	.	-1.43	0.57	.	*	.	-0.60	0.24
	Ser	132	.	.	B	B	.	.	-1.43	0.69	.	*	.	-0.60	0.35
15	Asn	133	.	A	B	.	.	.	-1.72	0.69	.	.	.	-0.60	0.21
	Val	134	.	A	B	.	.	.	-0.83	0.80	.	.	.	-0.60	0.31
	Ala	135	.	A	B	.	.	.	-0.53	0.11	.	.	.	-0.30	0.38
	Leu	136	A	A	.	.	.	.	0.01	-0.27	.	.	.	0.30	0.41
	Ala	137	A	A	.	.	.	.	0.31	-0.24	.	.	.	0.30	0.55
20	Asp	138	A	A	.	.	.	.	0.07	-0.89	.	.	F	0.75	0.95
	Glu	139	A	A	.	.	.	.	0.61	-0.63	.	.	F	0.90	1.80
	Gly	140	A	.	.	.	.	.	0.53	-0.83	*	.	F	1.10	2.57
	Glu	141	A	.	.	.	.	.	1.04	-0.76	*	*	F	0.95	0.82
	Tyr	142	A	.	.	.	.	T	0.74	-0.37	*	.	.	0.70	0.64
25	Thr	143	A	.	.	.	.	T	0.04	0.31	*	.	.	0.10	0.45
	Cys	144	.	.	B	.	.	T	-0.27	0.67	.	*	.	-0.20	0.23
	Ser	145	.	.	B	.	.	T	-0.52	1.16	*	.	.	-0.20	0.21
	Ile	146	.	.	B	B	.	.	-0.73	1.01	.	*	.	-0.60	0.14
	Phe	147	.	.	B	B	.	.	-1.34	0.96	*	*	.	-0.60	0.41
30	Thr	148	.	.	B	B	.	.	-0.92	1.03	*	*	.	-0.60	0.23
	Met	149	.	.	B	B	.	.	-0.57	0.64	*	*	.	-0.60	0.64
	Pro	150	.	.	B	B	.	.	-0.86	0.44	*	.	.	-0.45	1.06
	Val	151	A	.	.	B	.	.	0.08	0.16	*	.	.	-0.30	0.74
35	Arg	152	A	.	.	B	.	.	0.48	-0.33	*	*	F	0.60	1.50
	Thr	153	A	.	.	B	.	.	-0.02	-0.56	*	*	F	0.90	1.30
	Ala	154	A	.	.	B	.	.	-0.28	-0.30	*	*	F	0.60	1.45
	Lys	155	A	.	.	B	.	.	-0.38	-0.30	*	*	F	0.45	0.55
	Ser	156	.	.	B	B	.	.	-0.38	0.19	*	.	F	-0.15	0.55
	Leu	157	.	.	B	B	.	.	-1.30	0.34	*	.	.	-0.30	0.40
40	Val	158	.	.	B	B	.	.	-1.33	0.53	*	.	.	-0.60	0.17
	Thr	159	.	.	B	B	.	.	-1.63	0.96	*	.	.	-0.60	0.12
	Val	160	.	.	B	B	.	.	-1.89	1.26	*	.	.	-0.60	0.10
	Leu	161	.	.	B	B	.	.	-1.59	1.00	*	.	.	-0.60	0.22
	Gly	162	.	.	B	B	.	.	-0.73	0.76	*	.	.	-0.60	0.26
45	Ile	163	.	.	B	B	.	.	-0.09	0.27	*	.	F	-0.15	0.70
	Pro	164	.	.	B	B	.	C	-0.67	0.06	*	.	F	0.20	1.32
	Gln	165	.	.	B	.	.	.	-0.70	0.06	*	.	F	0.05	0.93
	Lys	166	.	.	B	B	.	.	-0.20	0.31	.	.	F	-0.15	0.93
	Pro	167	.	.	B	B	.	.	-0.20	0.11	.	.	F	-0.15	0.87
50	Ile	168	.	.	B	B	.	.	0.44	0.11	*	.	.	-0.30	0.50
	Ile	169	.	.	B	B	.	.	0.70	0.47	*	.	.	-0.60	0.39
	Thr	170	.	.	B	B	.	.	0.40	0.47	*	.	.	-0.60	0.50
	Gly	171	.	.	B	.	.	.	0.06	0.43	*	.	F	0.05	0.96
	Tyr	172	.	.	B	.	.	T	-0.54	0.13	*	*	F	1.00	1.84
55	Lys	173	.	.	.	.	.	T	0.46	0.13	*	*	F	1.50	1.05
	Ser	174	.	.	.	.	.	T	1.34	-0.36	*	*	F	2.40	2.08
	Ser	175	.	.	.	.	.	T	1.70	-0.79	*	*	F	3.00	2.30
	Leu	176	.	A	B	.	.	.	2.04	-1.54	*	*	F	2.10	2.30
	Arg	177	A	A	.	.	.	.	1.98	-1.54	*	*	F	1.80	2.87
60	Glu	178	A	A	.	.	.	.	1.34	-1.44	*	*	F	1.50	3.09
	Lys	179	A	A	.	.	.	.	1.33	-1.33	.	*	F	1.20	3.79
	Asp	180	A	A	.	.	.	.	0.82	-1.53	.	*	F	0.90	2.79
	Thr	181	A	A	.	.	.	.	1.63	-0.84	.	*	F	0.90	1.33
	Ala	182	A	A	.	.	.	.	0.86	-0.44	.	*	F	0.60	1.07

5	Thr	183	.	A	B	.	.	.	.	0.86	0.13	.	*	.	-0.30	0.34
	Leu	184	.	A	B	.	.	.	.	0.51	0.53	.	*	.	-0.60	0.41
	Asn	185	.	A	B	.	.	.	.	0.21	0.43	.	*	.	-0.60	0.55
	Cys	186	.	.	B	.	.	.	.	0.18	0.31	.	*	F	0.39	0.51
	Gln	187	.	.	.	.	T	.	.	0.47	0.26	.	*	F	1.13	0.61
10	Ser	188	.	.	.	.	T	T	.	0.82	-0.04	.	*	F	2.27	0.51
	Ser	189	.	.	.	.	T	T	.	1.42	-0.44	*	*	F	2.76	1.89
	Gly	190	.	.	.	.	T	T	.	0.83	-0.59	*	.	F	3.40	1.69
	Ser	191	.	.	.	.	.	T	C	0.91	-0.49	*	*	F	2.56	1.27
	Lys	192	.	A	.	.	.	.	C	1.02	-0.37	*	*	F	1.67	0.96
15	Pro	193	.	A	.	.	.	.	C	0.51	-0.76	*	*	F	1.78	1.90
	Ala	194	.	A	B	.	.	.	.	0.50	-0.50	*	*	F	1.24	1.17
	Ala	195	.	A	B	B	.	.	.	0.56	-0.40	*	*	.	0.30	0.84
	Arg	196	.	A	B	B	.	.	.	0.97	0.51	*	*	.	-0.60	0.57
	Leu	197	.	A	B	B	.	.	.	0.97	0.09	*	*	.	0.19	1.11
20	Thr	198	A	A	.	B	.	.	.	0.83	-0.41	*	*	.	1.13	2.20
	Trp	199	A	A	.	B	.	.	.	1.42	-0.49	*	*	.	1.47	1.11
	Arg	200	.	.	.	.	.	T	C	2.01	-0.49	*	*	F	2.56	2.25
	Lys	201	.	.	.	.	T	T	.	1.90	-0.77	*	*	F	3.40	2.70
	Gly	202	.	.	.	.	T	T	.	1.90	-1.26	*	*	F	3.06	4.45
25	Asp	203	.	.	.	.	.	T	C	2.18	-1.49	*	*	F	2.52	1.87
	Gln	204	.	A	.	.	.	.	C	2.12	-0.99	*	*	F	1.78	1.28
	Glu	205	.	A	.	.	.	.	C	2.01	-0.56	*	*	F	1.44	1.28
	Leu	206	.	A	.	.	.	.	C	1.76	-0.99	*	*	F	1.10	1.32
	His	207	.	A	.	.	T	.	.	1.79	-0.56	*	*	F	1.64	1.18
30	Gly	208	.	A	.	.	.	.	C	1.90	-0.47	*	*	F	1.33	0.98
	Glu	209	.	.	.	.	.	T	C	1.01	-0.47	*	*	F	2.22	2.34
	Pro	210	.	.	.	.	.	T	C	1.01	-0.47	*	*	F	2.56	1.20
	Thr	211	.	.	.	.	T	T	.	1.82	-0.57	*	*	F	3.40	2.11
	Arg	212	.	.	B	.	.	T	.	1.86	-1.00	*	*	F	2.66	2.11
35	Ile	213	.	.	B	.	.	.	.	1.99	-1.00	*	*	F	2.46	2.28
	Gln	214	.	.	B	.	.	.	.	1.99	-1.00	*	*	F	2.46	2.44
	Glu	215	.	.	B	.	.	.	.	1.86	-1.09	*	*	F	2.46	2.00
	Asp	216	.	.	.	.	.	T	C	2.21	-0.66	*	*	F	2.86	2.83
	Pro	217	.	.	.	.	T	T	.	1.79	-1.34	.	*	F	3.40	3.26
40	Asn	218	.	.	.	.	T	T	.	1.98	-1.26	.	*	F	3.06	2.72
	Gly	219	.	.	.	.	T	T	.	1.67	-0.47	.	*	F	2.42	1.41
	Lys	220	.	.	.	B	T	.	.	0.81	0.01	.	.	F	1.08	1.32
	Thr	221	.	.	B	B	.	.	.	0.51	0.23	.	.	F	0.19	0.61
	Phe	222	.	.	B	B	.	.	.	0.42	0.21	.	.	F	-0.15	0.82
45	Thr	223	.	.	B	B	.	.	.	0.12	0.17	.	*	.	-0.30	0.55
	Val	224	.	.	B	.	.	T	.	-0.39	0.56	.	.	F	-0.05	0.51
	Ser	225	.	.	B	.	.	T	.	-0.74	0.71	.	*	F	-0.05	0.44
	Ser	226	.	.	.	.	.	T	C	-1.13	0.41	.	*	F	0.15	0.44
	Ser	227	.	.	.	.	.	T	C	-0.43	0.71	.	*	F	0.15	0.51
50	Val	228	.	.	B	B	.	.	.	-0.98	0.47	.	*	F	-0.45	0.66
	Thr	229	.	.	B	B	.	.	.	-0.43	0.73	*	*	.	-0.60	0.37
	Phe	230	.	.	B	B	.	.	.	-0.02	0.83	*	*	.	-0.60	0.39
	Gln	231	.	.	B	B	.	.	.	0.28	0.44	*	*	.	-0.45	1.04
	Val	232	.	.	B	B	.	.	.	0.58	-0.20	*	*	.	0.79	1.25
55	Thr	233	.	.	B	B	.	.	.	1.43	-0.69	*	*	F	1.58	2.41
	Arg	234	.	.	B	B	.	.	.	1.40	-1.47	*	.	F	1.92	2.32
	Glu	235	.	.	.	B	T	.	.	1.51	-1.44	*	.	F	2.66	3.10
	Asp	236	.	.	.	.	T	T	.	1.21	-1.59	.	.	F	3.40	2.17
	Asp	237	.	.	.	.	T	T	.	1.18	-1.69	*	.	F	3.06	1.48
60	Gly	238	.	.	.	.	T	T	.	0.63	-1.00	.	.	F	2.57	0.60
	Ala	239	A	.	.	.	.	T	.	-0.14	-0.36	.	.	.	1.38	0.27
	Ser	240	.	.	B	B	.	.	.	-0.44	0.21	*	.	.	0.04	0.09
	Ile	241	.	.	B	B	.	.	.	-1.30	0.60	*	.	.	-0.60	0.12
	Val	242	.	.	B	B	.	.	.	-1.30	0.81	*	.	.	-0.60	0.09
60	Cys	243	.	.	B	B	.	.	.	-0.99	0.71	*	*	.	-0.60	0.10
	Ser	244	.	.	B	B	.	.	.	-0.40	0.83	.	*	.	-0.60	0.20
	Val	245	.	.	B	B	.	.	.	-0.40	0.14	.	.	.	-0.30	0.46

5	Asn	246	A	.	.	B	.	.	.	-0.32	-0.11	*	.	.	0.45	1.16
	His	247	A	A	.	.	.	.	.	0.58	0.00	*	.	.	0.30	0.71
	Glu	248	A	A	.	.	.	.	.	0.90	-0.39	*	.	F	0.60	1.92
	Ser	249	A	A	.	.	.	.	.	0.61	-0.60	*	.	F	0.90	1.18
	Leu	250	A	A	.	.	.	.	.	1.47	-0.50	*	*	F	0.75	0.88
10	Lys	251	A	A	.	.	.	.	.	1.58	-1.00	*	*	F	0.75	0.85
	Gly	252	A	.	.	.	.	T	.	1.31	-1.00	*	*	F	1.60	1.24
	Ala	253	A	.	.	.	.	T	.	1.00	-1.00	*	*	F	1.90	2.01
	Asp	254	A	.	.	.	.	T	.	1.00	-1.20	*	*	F	2.20	1.45
	Arg	255	A	.	.	.	.	T	.	1.81	-0.81	*	*	F	2.50	1.96
15	Ser	256	.	.	.	.	.	T	C	1.88	-0.84	*	*	F	3.00	3.37
	Thr	257	.	.	.	.	.	T	C	1.33	-1.34	*	*	F	2.70	3.95
	Ser	258	.	.	.	.	.	T	C	1.92	-0.66	*	*	F	2.40	1.41
	Gln	259	.	.	B	.	.	T	.	1.07	-0.66	*	*	F	1.90	1.83
	Arg	260	.	.	B	B	.	.	.	0.14	-0.40	*	*	F	0.75	0.94
20	Ile	261	.	.	B	B	.	.	.	0.20	-0.20	*	*	F	0.45	0.58
	Glu	262	.	.	B	B	.	.	.	0.20	0.17	.	*	.	-0.30	0.52
	Val	263	.	.	B	B	.	.	.	0.29	0.26	.	*	.	-0.30	0.39
	Leu	264	.	.	B	B	.	.	.	-0.02	0.69	*	*	.	-0.60	0.85
	Tyr	265	.	.	B	B	.	.	.	-0.72	0.49	.	*	.	-0.60	0.71
25	Thr	266	.	.	B	.	.	T	.	-0.43	0.99	*	*	.	-0.20	0.96
	Pro	267	.	.	.	.	.	T	C	-1.32	0.96	*	*	F	0.30	1.16
	Thr	268	.	.	B	.	.	T	.	-0.36	0.96	*	*	.	-0.20	0.52
	Ala	269	.	.	B	.	.	T	.	0.24	0.20	.	*	.	0.10	0.70
	Met	270	.	.	B	.	.	.	.	0.49	0.14	.	*	.	-0.10	0.70
30	Ile	271	.	.	B	.	.	.	.	0.59	-0.29	.	*	.	0.50	0.81
	Arg	272	.	.	B	.	.	T	.	0.59	-0.34	.	*	.	0.85	1.24
	Pro	273	.	.	.	.	T	T	.	0.87	-0.41	.	*	F	1.40	1.94
	Asp	274	.	.	.	.	.	T	C	1.24	-0.53	*	*	F	1.50	3.77
	Pro	275	.	.	.	.	.	T	C	1.96	-0.79	*	*	F	1.84	2.98
35	Pro	276	.	.	.	.	.	.	C	2.84	-0.79	*	*	F	1.98	3.77
	His	277	.	.	.	.	.	T	C	2.39	-1.21	*	.	F	2.52	3.91
	Pro	278	.	.	.	.	.	T	C	2.60	-0.79	*	*	F	2.86	2.50
	Arg	279	.	.	.	.	T	T	.	2.64	-0.81	*	*	F	3.40	2.80
	Glu	280	A	.	.	.	.	T	.	2.04	-1.24	*	.	F	2.66	4.12
40	Gly	281	A	A	.	.	.	.	.	1.44	-1.06	*	*	F	1.92	2.20
	Gln	282	A	A	.	.	.	.	.	0.67	-0.80	*	*	F	1.43	0.93
	Lys	283	A	A	.	.	.	.	.	0.84	-0.11	*	*	F	0.79	0.44
	Leu	284	A	A	.	.	.	.	.	0.07	0.39	*	*	F	-0.15	0.61
	Leu	285	.	A	B	.	.	.	.	0.07	0.53	*	*	.	-0.60	0.19
45	Leu	286	.	A	B	.	.	.	.	0.07	0.13	*	*	.	-0.30	0.16
	His	287	.	A	B	.	.	.	.	0.18	0.56	*	*	.	-0.26	0.19
	Cys	288	.	A	B	.	.	.	.	-0.21	-0.13	*	*	.	0.98	0.46
	Glu	289	.	A	.	.	T	.	.	0.60	-0.39	.	*	F	1.87	0.56
	Gly	290	.	.	.	.	T	T	.	1.20	-0.67	.	*	F	2.91	0.66
50	Arg	291	.	.	.	.	T	T	.	1.16	-0.74	.	*	F	3.40	1.89
	Gly	292	.	.	.	.	T	T	.	0.98	-0.67	.	*	F	2.91	0.81
	Asn	293	.	.	.	.	.	T	C	1.64	-0.24	.	*	F	2.22	1.27
	Pro	294	.	.	.	.	.	.	C	1.64	-0.27	.	*	F	1.68	1.12
	Val	295	.	.	.	.	.	.	C	1.74	0.13	*	*	F	0.74	1.96
55	Pro	296	.	.	B	.	.	.	.	0.82	0.46	*	*	F	-0.10	1.91
	Gln	297	.	A	B	.	.	.	.	0.88	0.74	.	.	F	-0.30	1.02
	Gln	298	.	A	B	.	.	.	.	0.88	1.23	.	.	F	-0.30	1.44
	Tyr	299	.	A	B	.	.	.	.	1.13	0.59	.	.	.	-0.45	1.62
	Leu	300	.	A	B	.	.	.	.	1.99	0.16	.	.	.	-0.15	1.87
60	Trp	301	.	A	B	.	.	.	.	1.86	-0.24	.	.	.	0.45	1.87
	Glu	302	.	A	B	.	.	.	.	1.56	-0.21	.	.	F	0.60	1.18
	Lys	303	.	A	.	.	T	.	.	0.70	-0.59	.	.	F	1.30	1.92
	Glu	304	.	A	.	.	T	.	.	0.73	-0.63	.	.	F	1.30	1.35
	Gly	305	.	A	.	.	T	.	.	1.33	-1.11	.	.	F	1.30	1.21
	Ser	306	.	.	.	.	.	.	C	0.81	-0.69	.	.	F	1.15	0.93
	Val	307	.	.	.	.	.	.	C	0.86	0.00	.	.	F	0.85	0.44
	Pro	308	.	.	.	.	.	T	C	0.21	0.00	.	.	F	1.05	0.90

5	Pro	309	A	.	.	.	.	T	C	-0.10	0.19	.	.	F	0.45	0.66
	Leu	310	A	.	.	.	.	T	.	0.24	0.29	.	.	F	0.40	1.29
	Lys	311	A	.	.	.	.	T	.	0.54	0.04	.	.	F	0.40	1.45
	Met	312	A	A	.	.	.	.	.	1.10	-0.39	*	.	F	0.60	1.62
	Thr	313	A	A	.	.	.	.	.	0.72	-0.43	.	*	F	0.60	2.63
10	Gln	314	A	A	.	.	.	.	.	0.12	-0.61	.	*	F	0.90	1.33
	Glu	315	A	A	.	.	.	.	.	0.04	0.07	*	*	F	0.00	1.11
	Ser	316	A	A	.	B	.	.	.	-0.70	0.14	*	.	F	-0.15	0.54
	Ala	317	A	A	.	B	.	.	.	-0.31	0.44	.	.	.	-0.60	0.27
	Leu	318	A	A	.	B	.	.	.	-0.70	0.47	.	.	.	-0.60	0.24
15	Ile	319	.	A	B	B	.	.	.	-1.51	1.26	.	.	.	-0.60	0.16
	Phe	320	.	A	B	B	.	.	.	-1.51	1.56	*	.	.	-0.60	0.13
	Pro	321	.	A	B	.	.	.	.	-1.17	1.46	*	.	.	-0.60	0.25
	Phe	322	.	.	B	.	.	.	.	-0.88	0.77	*	.	.	-0.40	0.70
	Leu	323	.	.	B	.	.	.	.	-0.07	0.47	*	.	.	0.09	1.09
20	Asn	324	.	.	.	.	T	.	.	0.52	-0.31	*	.	F	1.88	1.18
	Lys	325	.	.	.	.	T	.	.	0.88	-0.36	.	.	F	2.22	1.82
	Ser	326	.	.	.	.	T	.	.	0.78	-0.71	*	.	F	2.86	2.19
	Asp	327	.	.	.	.	T	T	.	1.23	-0.91	.	.	F	3.40	1.96
	Ser	328	.	.	.	.	T	T	.	1.70	-0.56	.	.	F	3.06	1.54
25	Gly	329	.	.	.	.	T	T	.	1.03	-0.13	.	.	F	2.42	1.14
	Thr	330	.	.	.	.	T	T	.	0.68	0.06	.	.	F	1.33	0.36
	Tyr	331	.	.	B	B	.	.	.	0.39	0.54	.	.	F	-0.11	0.39
	Gly	332	.	.	B	B	.	.	.	0.08	0.66	.	.	.	-0.60	0.40
	Cys	333	.	.	B	B	.	.	.	0.08	0.71	.	.	.	-0.60	0.40
30	Thr	334	.	.	B	B	.	.	.	0.42	0.61	.	.	.	-0.60	0.34
	Ala	335	.	.	B	B	.	.	.	0.13	0.26	.	.	F	-0.15	0.56
	Thr	336	.	.	B	B	.	.	.	0.03	0.44	.	.	F	-0.30	1.03
	Ser	337	.	.	B	B	.	.	.	0.08	0.30	.	.	F	-0.06	0.71
	Asn	338	.	.	B	.	T	T	.	0.50	0.20	.	.	F	0.83	0.94
35	Met	339	.	.	.	.	T	T	.	0.86	0.46	.	.	F	0.77	1.02
	Gly	340	.	.	.	.	T	T	.	0.86	-0.03	.	.	F	1.76	1.52
	Ser	341	.	.	.	.	.	T	C	0.92	0.09	.	.	F	0.90	0.95
	Tyr	342	.	.	B	B	.	.	.	0.98	0.44	.	.	.	-0.09	1.51
	Lys	343	.	.	B	B	.	.	.	0.67	0.59	.	*	.	-0.18	2.39
40	Ala	344	.	.	B	B	.	.	.	0.46	0.64	.	*	.	-0.27	2.57
	Tyr	345	.	.	B	B	.	.	.	0.80	0.94	.	*	.	-0.36	1.35
	Tyr	346	.	.	B	B	.	.	.	0.24	0.59	.	*	.	-0.45	1.09
	Thr	347	.	.	B	B	.	.	.	0.49	1.23	.	*	.	-0.60	0.80
	Leu	348	.	.	B	B	.	.	.	0.44	1.13	.	*	.	-0.36	0.82
45	Asn	349	.	.	B	B	.	.	.	0.82	0.37	.	*	.	0.18	0.87
	Val	350	.	.	B	B	.	.	.	0.77	0.04	.	*	.	0.42	0.94
	Asn	351	.	.	.	B	T	.	.	0.80	-0.06	.	*	F	1.96	1.52
	Asp	352	.	.	.	.	.	T	C	0.26	-0.31	.	*	F	2.40	1.46
	Pro	353	.	.	B	.	.	T	.	0.86	-0.07	.	*	F	1.96	1.46
50	Ser	354	.	.	.	.	.	T	C	0.56	-0.29	.	.	F	1.92	1.41
	Pro	355	.	.	B	.	.	T	.	1.11	-0.30	.	.	F	1.48	1.13
	Val	356	.	.	B	.	.	T	.	0.81	0.09	.	.	F	0.49	0.98
	Pro	357	.	.	B	.	.	T	.	0.51	0.04	.	.	F	0.25	0.98
	Ser	358	.	.	.	.	T	T	.	0.41	0.04	.	.	F	0.65	0.85
55	Ser	359	.	.	B	.	.	T	.	0.47	0.10	.	.	F	0.40	1.65
	Ser	360	.	.	B	.	.	T	.	0.64	0.21	.	.	F	0.40	1.67
	Ser	361	.	.	B	.	.	T	.	0.91	0.29	.	.	F	0.40	1.70
	Thr	362	.	.	B	.	.	T	.	0.23	0.40	.	.	F	0.40	1.28
	Tyr	363	.	.	B	.	.	T	.	-0.36	0.70	.	.	.	-0.20	0.67
60	His	364	.	.	B	B	.	.	.	-0.40	1.00	.	.	.	-0.60	0.35
	Ala	365	.	.	B	B	.	.	.	-0.44	1.04	*	.	.	-0.60	0.24
	Ile	366	.	.	B	B	.	.	.	-1.03	0.99	*	.	.	-0.60	0.15
	Ile	367	.	.	B	B	.	.	.	-1.58	0.91	.	.	.	-0.60	0.08
	Gly	368	.	.	B	B	.	.	.	-1.92	1.06	*	.	.	-0.60	0.06
60	Gly	369	.	.	B	B	.	.	.	-2.59	1.06	*	.	.	-0.60	0.08
	Ile	370	.	.	B	B	.	.	.	-2.89	1.16	.	.	.	-0.60	0.10
	Val	371	.	.	B	B	.	.	.	-2.86	1.16	.	.	.	-0.60	0.07

5	Ala	372	.	.	B	B	.	.	.	-2.67	1.37	.	.	.	-0.60	0.05
	Phe	373	.	.	B	B	.	.	.	-3.13	1.73	.	.	.	-0.60	0.07
	Ile	374	.	.	B	B	.	.	.	-3.60	1.73	.	.	.	-0.60	0.07
	Val	375	.	.	B	B	.	.	.	-3.52	1.77	.	.	.	-0.60	0.06
	Phe	376	A	.	.	B	.	.	.	-3.56	1.96	.	.	.	-0.60	0.06
10	Leu	377	A	.	.	B	.	.	.	-3.57	1.86	.	.	.	-0.60	0.06
	Leu	378	A	.	.	B	.	.	.	-3.68	1.79	.	.	.	-0.60	0.08
	Leu	379	A	.	.	B	.	.	.	-3.68	1.83	.	.	.	-0.60	0.07
	Ile	380	A	.	.	B	.	.	.	-3.52	1.73	.	.	.	-0.60	0.06
	Met	381	A	.	.	B	.	.	.	-3.63	1.83	.	.	.	-0.60	0.07
15	Leu	382	A	.	.	B	.	.	.	-3.17	1.83	.	.	.	-0.60	0.07
	Ile	383	A	.	.	B	.	.	.	-2.39	1.57	.	.	.	-0.60	0.09
	Phe	384	A	.	.	B	.	.	.	-1.82	1.39	.	.	.	-0.60	0.13
	Leu	385	A	.	.	B	.	.	.	-1.74	1.53	.	.	.	-0.60	0.24
	Gly	386	A	.	.	B	.	.	.	-2.03	1.53	*	*	.	-0.60	0.28
20	His	387	A	.	.	B	.	.	.	-1.11	1.53	*	*	.	-0.60	0.23
	Tyr	388	A	.	.	B	.	.	.	-0.26	0.74	.	.	.	-0.60	0.55
	Leu	389	.	.	B	B	.	.	.	0.49	0.56	.	*	.	-0.32	0.75
	Ile	390	.	.	B	B	.	.	.	0.96	0.13	*	*	.	0.41	1.11
	Arg	391	.	.	B	B	.	.	.	0.99	0.06	*	*	.	0.54	0.70
25	His	392	.	.	.	.	T	T	.	0.78	-0.21	*	*	.	2.37	1.22
	Lys	393	.	.	.	.	T	T	.	0.21	-0.14	*	*	F	2.80	2.73
	Gly	394	.	.	.	.	.	T	C	0.71	-0.14	*	*	F	2.32	1.15
	Thr	395	.	.	.	.	.	T	C	1.57	0.34	*	*	F	1.44	1.22
	Tyr	396	.	.	B	.	.	.	.	1.46	0.34	.	*	.	0.46	0.83
30	Leu	397	.	A	B	.	.	.	.	0.90	0.34	*	*	.	0.13	1.45
	Thr	398	.	A	B	.	.	.	.	0.90	0.41	.	*	.	-0.45	1.02
	His	399	A	A	.	.	.	.	.	0.90	-0.07	*	*	.	0.79	1.30
	Glu	400	A	A	.	.	.	.	.	0.91	-0.40	*	*	.	1.13	1.56
	Ala	401	A	A	.	.	.	.	.	1.16	-0.70	*	*	F	1.92	1.45
35	Lys	402	.	A	.	.	T	.	.	1.97	-1.19	*	*	F	2.66	1.78
	Gly	403	.	.	.	.	T	T	.	1.69	-1.69	*	*	F	3.40	1.71
	Ser	404	.	.	.	.	.	T	C	1.51	-1.19	*	*	F	2.86	1.71
	Asp	405	.	.	.	.	T	T	.	1.51	-1.26	*	*	F	2.72	1.32
	Asp	406	A	.	.	.	.	T	C	1.51	-1.26	*	*	F	2.18	2.23
40	Ala	407	A	.	.	.	.	.	.	1.47	-1.19	*	.	F	1.44	1.68
	Pro	408	A	.	.	.	.	.	.	1.50	-1.57	.	.	F	1.10	1.68
	Asp	409	A	.	.	.	.	T	.	1.21	-1.09	*	.	F	1.30	1.46
	Ala	410	A	.	.	.	.	T	.	0.32	-0.59	*	.	F	1.30	1.46
	Asp	411	A	.	.	.	.	T	.	-0.57	-0.40	*	.	F	0.85	0.66
45	Thr	412	A	.	.	.	.	T	.	0.02	-0.14	*	.	F	0.85	0.28
	Ala	413	A	.	.	B	.	.	.	-0.36	0.26	.	*	.	-0.30	0.44
	Ile	414	.	.	B	B	.	.	.	-0.36	0.26	.	*	.	-0.30	0.27
	Ile	415	.	.	B	B	.	.	.	-0.11	0.26	.	.	.	-0.30	0.32
	Asn	416	.	.	B	.	.	T	.	-0.46	0.20	.	.	.	0.10	0.31
50	Ala	417	.	.	B	.	.	T	.	-0.14	0.13	.	.	F	0.25	0.44
	Glu	418	.	.	.	.	T	T	.	0.14	-0.16	.	.	F	1.40	1.09
	Gly	419	.	.	.	.	T	T	.	0.69	-0.46	.	.	F	1.55	0.91
	Gly	420	.	.	.	.	T	.	.	1.23	-0.43	.	*	F	1.65	0.89
	Gln	421	.	.	.	.	.	T	C	1.23	-0.50	*	.	F	2.25	0.51
55	Ser	422	.	.	.	.	.	T	C	1.82	-0.50	.	.	F	2.55	0.86
	Gly	423	.	.	.	.	.	T	C	1.87	-0.93	*	.	F	3.00	1.45
	Gly	424	.	.	.	.	.	T	C	2.26	-1.36	*	.	F	2.70	1.68
	Asp	425	.	.	.	.	.	T	C	2.60	-1.76	*	.	F	2.58	2.50
	Asp	426	.	.	.	.	.	T	C	2.36	-2.14	*	.	F	2.46	4.38
60	Lys	427	A	.	.	.	.	T	.	1.96	-1.81	.	.	F	2.14	6.94
	Lys	428	A	.	.	.	.	T	.	1.41	-1.46	.	.	F	2.02	3.60
	Glu	429	.	.	B	B	.	.	.	1.37	-0.77	.	.	F	1.80	1.51
	Tyr	430	.	.	B	B	.	.	.	0.98	-0.34	.	.	.	1.02	0.97
	Phe	431	.	.	B	B	.	.	.	0.59	0.09	.	.	.	0.24	0.62
	Ile	432	A	.	.	B	.	.	.	0.16	0.51	.	.	.	-0.24	0.46



**Table 5**

(Gene No:62 / Clone ID HEMAE80)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	.	.	0.59	-0.19	.	*	.	0.86	1.62
Arg	2	.	.	B	.	.	.	.	0.77	-0.19	.	*	.	1.07	1.25
Thr	3	.	.	.	.	.	T	C	0.34	-0.19	.	*	.	1.68	1.52
Pro	4	.	.	.	.	.	T	C	0.52	0.07	.	*	.	1.29	1.26
Gly	5	.	.	.	.	.	T	C	0.06	-0.11	.	*	F	2.10	1.00
Pro	6	.	.	.	.	.	T	C	-0.16	0.53	.	*	F	0.99	0.51
Leu	7	.	A	B	.	.	.	.	-1.08	0.73	.	*	F	0.18	0.27
Pro	8	.	A	B	.	.	.	.	-1.58	0.99	.	.	.	-0.18	0.23
Val	9	.	A	B	.	.	.	.	-2.18	1.24	.	.	.	-0.39	0.12
Leu	10	.	A	B	.	.	.	.	-2.64	1.50	.	.	.	-0.60	0.12
Leu	11	.	A	B	.	.	.	.	-3.02	1.50	.	.	.	-0.60	0.06
Leu	12	.	A	B	.	.	.	.	-2.56	1.57	.	.	.	-0.60	0.09
Leu	13	.	A	B	.	.	.	.	-2.93	1.36	.	.	.	-0.60	0.11
Leu	14	.	A	B	.	.	.	.	-2.29	1.17	.	.	.	-0.60	0.13
Ala	15	.	A	B	.	.	.	.	-2.07	0.91	.	.	.	-0.60	0.24
Gly	16	.	A	B	.	.	.	.	-1.84	0.73	.	.	.	-0.60	0.30
Ala	17	.	.	B	.	.	.	.	-0.92	0.54	.	.	.	-0.40	0.37
Pro	18	.	.	B	.	.	.	.	-0.32	-0.14	.	.	.	0.74	0.71
Ala	19	.	.	.	.	T	.	.	0.18	-0.21	.	.	.	1.53	1.11
Ala	20	.	.	B	.	.	.	.	0.56	-0.16	.	.	.	1.37	1.58
Arg	21	.	.	B	.	.	.	.	0.69	-0.23	.	.	F	1.76	1.58
Pro	22	.	.	.	.	T	.	.	0.97	-0.23	.	.	F	2.40	2.42
Thr	23	.	.	.	.	.	.	C	0.51	-0.24	.	.	F	1.96	3.46
Pro	24	.	.	.	.	.	T	C	0.86	-0.17	.	.	F	1.77	0.95
Pro	25	.	.	.	.	T	T	.	1.14	0.59	.	*	F	0.83	0.96
Thr	26	.	.	.	.	T	T	.	1.14	0.54	.	*	F	0.59	0.89
Cys	27	.	.	B	.	.	T	.	0.76	0.06	.	*	.	0.25	1.13
Tyr	28	.	.	B	.	.	.	.	1.18	0.24	.	*	.	-0.10	0.72
Ser	29	.	A	B	.	.	.	.	0.80	-0.19	.	*	.	0.30	0.98
Arg	30	.	A	B	.	.	.	.	0.20	-0.17	.	*	.	0.45	1.85
Met	31	.	A	B	.	.	.	.	0.21	-0.06	.	*	.	0.30	0.97
Arg	32	.	A	B	.	.	.	.	0.88	-0.43	.	*	.	0.30	0.97
Ala	33	.	A	B	.	.	.	.	1.12	-0.41	*	*	.	0.30	0.86
Leu	34	.	A	.	.	.	.	C	0.53	-0.41	*	*	.	0.65	1.50
Ser	35	.	A	B	.	.	.	.	0.11	-0.34	*	*	F	0.45	0.54
Gln	36	.	A	B	.	.	.	.	0.82	0.14	*	.	F	-0.15	0.77
Glu	37	.	A	B	.	.	.	.	0.71	-0.36	*	.	F	0.60	1.83
Ile	38	.	A	B	.	.	.	.	0.60	-1.04	*	.	F	0.90	2.28
Thr	39	.	A	B	.	.	.	.	1.41	-0.64	*	*	F	0.90	1.14
Arg	40	.	A	B	.	.	.	.	0.90	-0.64	*	.	F	0.90	1.06
Asp	41	.	A	.	.	T	.	.	0.09	0.04	*	.	F	0.40	1.24
Phe	42	.	.	B	B	.	.	.	0.09	0.04	*	.	.	-0.30	0.71
Asn	43	.	.	B	B	.	.	.	0.12	-0.04	*	.	.	0.30	0.63
Leu	44	.	.	.	B	.	.	C	0.13	0.60	.	.	.	-0.40	0.28
Leu	45	.	.	B	B	.	.	.	0.02	0.99	.	.	.	-0.60	0.43
Gln	46	.	.	B	B	.	.	.	-0.19	0.20	.	.	.	0.04	0.47
Val	47	.	.	.	B	.	.	C	0.21	0.23	.	.	.	0.58	0.87
Ser	48	.	.	.	B	.	.	C	0.21	-0.07	.	.	F	1.82	1.42
Glu	49	.	.	.	.	.	T	C	0.81	-0.76	.	.	F	2.86	1.42
Pro	50	.	.	.	.	T	T	.	0.96	-0.73	.	.	F	3.40	2.95
Ser	51	.	.	.	.	T	T	.	0.10	-0.80	*	*	F	3.06	1.18
Glu	52	.	.	.	.	.	T	C	1.07	-0.54	*	*	F	2.37	0.51
Pro	53	.	.	.	B	T	.	.	1.12	-0.54	*	.	F	1.83	0.64
Cys	54	.	.	B	B	.	.	.	0.31	-0.21	*	.	.	0.64	0.75
Val	55	.	.	B	B	.	.	.	0.31	0.09	*	*	.	-0.30	0.36
Arg	56	.	.	B	B	.	.	.	0.72	0.51	*	*	.	-0.60	0.36
Tyr	57	.	.	B	B	.	.	.	-0.09	0.09	*	*	.	-0.15	1.30
Leu	58	.	.	B	B	.	.	.	-0.12	0.20	*	*	.	-0.15	1.45

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Pro	59	.	.	B	B	.	.	-0.27	0.31	*	*	.	-0.15	1.16
Arg	60	.	.	B	B	.	.	0.59	1.00	*	*	.	-0.60	0.61
Leu	61	.	.	B	B	.	.	-0.41	0.24	*	*	.	-0.15	1.24
Tyr	62	.	.	B	B	.	.	-0.20	0.24	*	*	.	-0.30	0.56
Leu	63	.	.	B	B	.	.	0.61	0.31	*	*	.	-0.30	0.39
Asp	64	.	.	B	B	.	.	0.58	0.71	*	*	.	-0.60	0.76
Ile	65	.	.	B	B	.	.	-0.20	0.79	*	*	.	-0.60	0.76
His	66	.	.	B	.	.	T	-0.24	0.60	.	*	.	-0.20	0.49
Asn	67	.	.	B	.	.	T	-0.81	0.56	.	*	.	-0.20	0.22
Tyr	68	.	.	B	.	.	T	0.00	1.24	.	*	.	-0.20	0.26
Cys	69	.	.	B	.	.	T	0.04	0.56	.	.	.	-0.20	0.32
Val	70	.	A	B	B	.	.	0.12	0.06	.	*	.	-0.30	0.39
Leu	71	.	A	B	B	.	.	0.27	0.34	*	*	.	-0.30	0.21
Asp	72	.	A	B	B	.	.	0.27	-0.41	*	.	F	0.45	0.76
Lys	73	.	A	B	.	.	.	-0.19	-0.99	*	*	F	0.90	1.70
Leu	74	.	A	B	B	.	.	-0.38	-0.84	*	.	F	0.90	1.79
Arg	75	.	A	B	B	.	.	-0.11	-0.89	*	.	F	0.75	0.79
Asp	76	.	A	B	B	.	.	0.40	-0.39	*	.	.	0.30	0.40
Phe	77	.	A	B	B	.	.	0.19	0.00	*	.	.	0.30	0.65
Val	78	.	A	B	B	.	.	-0.07	-0.26	*	*	.	0.30	0.52
Ala	79	.	A	B	B	.	.	0.08	0.17	*	.	.	-0.30	0.48
Ser	80	.	A	.	B	.	.	-0.32	0.74	.	.	.	-0.40	0.30
Pro	81	.	.	.	.	.	T	-0.28	0.87	.	*	F	0.15	0.42
Pro	82	.	.	.	.	T	T	-0.43	0.23	.	.	F	0.65	0.83
Cys	83	.	.	.	.	T	T	-0.17	0.37	.	.	.	0.50	0.46
Trp	84	.	.	.	.	T	T	0.42	0.49	.	.	.	0.20	0.30
Lys	85	.	A	B	.	.	.	-0.13	0.46	.	.	.	-0.60	0.34
Val	86	.	A	B	.	.	.	0.08	0.67	.	.	.	-0.60	0.46
Ala	87	.	A	B	.	.	.	-0.01	0.10	.	.	.	-0.30	0.74
Gln	88	.	A	B	.	.	.	-0.16	-0.43	.	.	.	0.30	0.49
Val	89	.	A	B	.	.	.	0.18	0.26	.	.	.	-0.30	0.55
Asp	90	.	A	B	.	.	.	0.13	-0.39	.	.	F	0.60	1.09
Ser	91	.	A	B	.	.	.	1.03	-0.89	.	.	F	0.90	1.05
Leu	92	A	A	.	.	.	.	1.03	-1.29	*	*	F	0.90	2.83
Lys	93	A	A	.	.	.	.	1.14	-1.43	*	*	F	0.90	1.71
Asp	94	.	A	.	.	T	.	2.04	-1.43	*	.	F	1.30	2.50
Lys	95	A	A	.	.	.	.	1.23	-1.81	*	*	F	0.90	6.06
Ala	96	A	A	.	.	.	.	1.29	-1.81	*	*	F	0.90	2.50
Arg	97	.	A	B	.	.	.	1.79	-1.06	*	*	F	0.90	2.34
Lys	98	.	A	B	.	.	.	0.86	-0.57	*	*	F	0.90	1.69
Leu	99	.	A	B	.	.	.	0.26	0.11	*	.	.	-0.15	1.17
Tyr	100	.	A	B	.	.	.	0.21	0.23	*	.	.	-0.30	0.59
Thr	101	.	.	B	B	.	.	0.50	0.63	*	.	.	-0.60	0.48
Ile	102	.	.	B	B	.	.	-0.31	1.01	*	.	.	-0.60	0.77
Met	103	.	.	B	B	.	.	-1.02	1.11	*	.	.	-0.60	0.43
Asn	104	.	.	B	.	.	T	-0.10	0.93	*	.	.	0.04	0.16
Ser	105	.	.	B	.	.	T	0.26	0.44	*	.	.	0.28	0.44
Phe	106	.	.	B	.	.	T	0.57	-0.24	*	.	.	1.42	0.88
Cys	107	.	.	B	.	.	T	0.64	-0.86	.	.	.	1.96	0.91
Arg	108	.	.	.	.	T	.	0.39	-0.57	.	.	.	2.40	0.56
Arg	109	.	.	B	B	.	.	-0.31	-0.31	*	.	F	1.41	0.48
Asp	110	.	.	B	B	.	.	-0.82	-0.31	.	.	F	1.17	0.78
Leu	111	.	.	B	B	.	.	-0.93	-0.20	*	.	.	0.78	0.33
Val	112	.	.	B	B	.	.	-0.27	0.49	*	.	.	-0.36	0.14
Phe	113	.	.	B	B	.	.	-0.38	0.49	*	.	.	-0.60	0.14
Leu	114	.	.	B	B	.	.	-1.16	0.49	*	.	.	-0.60	0.28
Leu	115	.	.	B	B	.	.	-1.16	0.37	.	.	.	-0.02	0.20
Asp	116	.	.	.	.	T	T	-0.93	0.13	.	.	F	1.21	0.37
Asp	117	.	.	.	.	T	T	-0.89	-0.16	.	.	F	2.09	0.46
Cys	118	.	.	.	.	T	T	-0.19	-0.16	.	.	.	2.22	0.46
Asn	119	.	.	.	.	T	T	0.38	-0.84	.	.	.	2.80	0.48
Ala	120	.	A	B	.	.	.	0.98	-0.09	.	.	.	1.42	0.45
Leu	121	.	A	B	.	.	.	0.09	0.34	.	.	.	0.69	1.29

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Glu	122	.	A	B	.	.	.	.	-0.12	0.46	.	*	.	-0.04	0.56
Tyr	123	.	A	B	.	.	.	.	-0.31	0.49	.	*	.	-0.32	0.86
Pro	124	.	.	B	B	.	.	.	-0.62	0.63	.	*	.	-0.60	0.77
Ile	125	.	.	B	B	.	.	.	-0.34	0.43	.	*	.	-0.60	0.64
Pro	126	.	.	B	B	.	.	.	-0.39	0.91	.	*	.	-0.60	0.59
Val	127	.	.	B	B	.	.	.	-1.20	0.80	.	.	.	-0.60	0.29
Thr	128	.	.	B	B	.	.	.	-1.17	1.06	.	.	.	-0.60	0.34
Thr	129	.	.	B	B	.	.	.	-0.96	0.80	.	.	F	-0.11	0.34
Val	130	.	.	B	B	.	.	.	0.04	0.37	.	.	F	0.53	0.75
Leu	131	.	.	B	.	.	T	.	0.26	-0.27	.	*	F	2.02	1.02
Pro	132	.	.	B	.	.	T	.	1.22	-0.36	.	*	F	2.36	1.23
Asp	133	.	.	.	.	T	T	.	1.14	-0.84	.	*	F	3.40	3.24
Arg	134	.	.	B	.	.	T	.	1.07	-1.06	.	*	.	2.51	5.03
Gln	135	.	.	B	.	.	.	.	1.53	-1.31	.	*	.	1.97	4.16
Arg	136	.	.	B	.	.	.	.	1.96	-1.31	.	*	.	1.63	3.18

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